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(57) Abstract

The present invention relates to methods for gene therapy, especially to adenovirus–based gene therapy, and related cell lines and compositions. In particular, novel nucleic acid constructs and packaging cell lines are disclosed, for use in facilitating the development of high–capacity and targeted vectors. The invention also discloses a variety of high–capacity adenovirus vectors and related compositions and kits including the disclosed cell lines and vectors. Finally, the invention discloses methods of preparing and using the disclosed vectors, cell lines and kits.

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Adenovirus Vectors, Packaging Cell Lines, Compositions, and Methods for Preparation and Use

This application is a continuation-in-part of U.S. Application 09/423,783 filed November 12, 1999 and claims the benefit of the filing date of U.S. Provisional Application 60/115,920 filed January 14, 1999.

This invention was made with U.S. government support under NIH Grant No. HL 54352. The government has certain rights in the invention.

The present invention relates to gene therapy, especially to adenovirus-based gene therapy. In particular, novel packaging cell lines are disclosed, for use in facilitating the development of high-capacity and targeted vectors. High-capacity adenovirus vectors are also disclosed herein, as are related compositions, kits, and methods of preparation and use of the disclosed vectors, cell lines and kits.

Enhanced transfer of DNA conjugates into cells has been achieved with adenovirus, a human DNA virus which readily infects epithelial cells (Horwitz, "Adenoviridae and Their Replication", in *Virology,* Fields and Knipe, eds., Raven Press, NY (1990) pp. 1679-1740).

There is a need in the art to obtain Adenovirus vectors capable of incorporating large segments of foreign DNA and capable of being targeted to specific cells, as well as to obtain cell lines which can package such adenovirus-gene deficient vectors or targeted vectors. These needs, as well as others, are met by the invention.

This invention utilizes recombinant adenovirus constructs which duplicate the cell receptor binding and DNA delivery properties of intact adenovirus virions and thus represents an improved method for gene therapy and cell targeting as well as for antisense-based antiviral therapy.

In contrast to the disadvantages of using intact adenovirus, modified adenovirus vectors requiring a helper plasmid or virus, or so-called replication-deficient adenovirus in the art, the use of recombinant adenovirus-derived vectors according to one aspect of the present invention provides certain advantages for gene delivery. First, the Ad-derived vectors of the present invention possess all of the functional properties required for gene therapy including binding to epithelial cell receptors and penetration of endocytic vesicles. Therapeutic viral vectors of the present invention may also be engineered to target the receptors of and

achieve penetration of non-epithelial cells; means of engineering viral vectors to accomplish these ends are described in detail herein below.

Second, the vectors of the present invention have deletions of substantial portions of the Ad genome, which not only limits the ability of the Ad-derived vectors to "spread" to other host cells or tissues, but allows significant amounts of "foreign" (or non-native) nucleic acids to be incorporated into the viral genome without interfering with the reproduction and packaging of the viral genome. Therefore, the vectors of the present invention are ideal for use in a wide variety of therapeutic applications.

Third, while the vectors disclosed herein are safe for use as therapeutic agents in the treatment of a variety of human afflictions, some of these vectors do not require the presence of any "helpers" for propagation and packaging, largely because of the novel cell lines in which they are reproduced. Such cell lines -- referred to herein as packaging cell lines -- comprise yet another aspect of the invention.

To reduce the frequency of contamination with wild-type adenovirus, it is desirable to improve either the viral vector or the cell line to reduce the probability of recombination. For example, an adenovirus from a group with less homology to the group C viruses may be used to engineer recombinant viruses with little propensity for recombination with the Ad5 sequence contained in the packaging lines. The invention describes the preparation of packaging cells lines which stably expresses adenovirus proteins or polypeptides. These cell lines are useful for complementing viral vectors bearing deletions of regulatory and/or structural genes, irrespective of the serotype from which such a vector was derived.

It is also contemplated that the constructs and methods of the present invention will support the design and engineering of chimeric viral vectors which express amino acid residue sequences derived from two or more Ad serotypes. Thus, unlike methods and constructs available prior to the advent of the present disclosure, this invention allows the greatest possible flexibility in the design and preparation of useful viral vectors and cell lines which support their construction and propagation -- all with a decreased risk of recombining with wild-type Ad to produce potentially-harmful recombinants.

In part, the present invention discloses a simpler, alternative means of reducing the recombination between viral and cellular sequences than those discussed in the art. One such means is to increase the size of the deletion in the recombinant virus and thereby reduce the extent of shared sequences between

that virus and any Ad genes present in a packaging cell line *e.g.*, the Ad5 genes in 293 cells, or the various Ad genes in the novel cell lines of the present invention.

Deletions of all or portions of structural genes of the adenovirus have been considered undesirable because of the anticipated deleterious effects such deletions would have on viral reproduction and packaging. Indeed, the use of "helper" viruses or plasmids has often been recommended when using Ad-derived vectors containing large deletions in structural protein sequences precisely for this reason.

Contrary to what has been suggested in the art, however, this invention discloses the preparation, propagation and use of recombinant Ad-derived vectors having deletions of all or part of various gene sequences encoding Ad structural proteins, both as a way of reducing the risk of wild-type adenovirus contamination in virus preparations, as a way of allowing foreign DNA to be packaged in such vectors for a variety of diagnostic and therapeutic applications and as a way of targeting an adenovirus vector to a specific cell type.

The invention further discloses a wide variety of nucleic acid sequences and viral vectors. Thus, in one embodiment, the invention discloses a nucleic acid sequence encoding any one of the adenovirus fiber proteins mentioned in the specification, polypeptides or fragments thereof -- including, without limitation, those that include deletions or other mutations; those that are chimeric; and those that have linkers, foreign amino acid residues, or other molecules attached for various purposes as disclosed herein. Nucleic acid sequences encoding various other adenovirus structural and/or regulatory proteins or polypeptides are also within the scope of the present invention.

In various embodiments, the adenovirus is a Group C adenovirus selected from serotypes 1, 2, 5 or 6; while in other embodiments, adenovirus selected from other serotypes, such as for example Ad37 (subgroup D) are useful as disclosed herein.

The invention is also directed to an isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide sequence, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third same or different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3. A preferable embodiment of the invention may further comprise an intron operatively linked to the TPL, wherein said intron also

contains requisite processing signals for the intron's removal. Another preferable embodiment of the invention is directed to the isolated nucleic acid molecule wherein said TPL nucleotide sequence consists essentially of complete TPL exon 1 operatively linked to complete TPL exon 2 operatively linked to complete TPL exon 3. A related embodiment may further include an intron and appropriate processing signals. Additional embodiments of the invention are directed to nucleic acid molecules contained in plasmids selected from the group; consisting of pCLF, pDV60, pDV67, pDV69, pDV80 and PDV90. Packaging cell lines and adenovirus particles containing the nucleic acids described above are also included in the invention.

The invention is further directed to methods for producing an adenovirus vector particle containing a helper-independent fiberless recombinant adenovirus vector genome comprising providing a) a packaging cell line which complements replication and packaging of said genome and b) a helper-independent fiberless recombinant adenovirus vector genome which is deficient in expressing sufficient functional fiber protein to support assembly of fiber-containing particles. The genome is introduced into the cell line. Additional embodiments of the invention may also include the following steps; a) growing the cell line produced under conditions for producing particles; and/or b) harvesting an adenovirus vector particle containing said helper-independent fiberless recombinant adenovirus vector genome. The method may also include a cell line that expresses a fiber protein and complements a fiber mutation in the vector.

The invention is also directed to an adenovirus vector packaging cell line comprising a stably integrated nucleic acid molecule as described above, an operatively-linked promoter and a nucleic acid sequence which encodes an adenovirus structural protein, wherein said TPL sequence consists essentially of a first TPL exon operatively linked to a complete second TPL exon operatively linked to a complete third TPL exon, wherein said packaging cell comprises an isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3. Preferably, the cell line may have a complete first TPL exon. Another embodiment of the invention comprises adenovirus

structural protein, such as adenovirus fiber protein or a chimeric protein which includes an adenovirus fiber protein tail domain.

The invention is further directed to a recombinant adenovirus particle comprising a recombinant adenovirus vector genome wherein said genome:(a) does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene, and (b) encodes an adenovirus packaging signal and inverted terminal repeats containing adenovirus origin of replication. The invention is also directed to a helper-independent fiberless recombinant adenovirus vector genome comprising genes which (a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene and (b) encodes an exogenous protein. Either of the above embodiments may substitute a helper-dependent for a helper-independent recombinant adenovirus vector genome. In a preferable embodiment, no fiber protein is expressed. In yet another embodiment of the invention, the recombinant adenovirus particle fails to express sufficient fiber protein to allow fiber incorporation into the particle such that the particle can use the fiber pathway for infection.

The invention is further directed to a method for producing an adenovirus vector particle containing a helper-independent fiberless recombinant adenovirus vector genome, said method comprising providing a packaging cell line which complements replication and packaging of said genome and a helper-independent fiberless recombinant adenovirus vector genome which is deficient in expressing sufficient functional fiber protein to support assembly of fiber-containing particles, wherein said packaging cell comprises an isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon and harvesting said adenovirus particles produced by said cell line.. In a preferable embodiment the adenovirus particle further comprises an exogenous protein or a modified fiber protein. The method may also comprise a step of coating a particle (i.e. providing fiber protein in any way) with an adenovirus fiber protein.

Another aspect of the invention is directed to a method for pseudotyping recombinant viral vectors comprising complementing a missing fiber gene of a helper-independent fiberless recombinant adenovirus vector genome by expressing in packaging cells a fiber gene from a different adenoviral serotype than said recombinant adenovirus vector, thereby pseudotyping said vector. An additional embodiment of the invention is directed to the method for pseudotyping recombinant viral vectors comprising: a) providing a packaging cell line for propagating a fiber gene deleted recombinant adenovirus vector, b) introducing into said cell line a helper-independent fiberless recombinant adenovirus vector genome, and c) complementing the missing fiber gene by expression in the cells of a fiber gene from a different adenoviral serotype thereby pseudotyping the vector.

The invention is further directed to a method for specifically targeting an adenovirus vector to a cell of choice comprising introducing a helper-independent or helper-dependent fiberless recombinant adenovirus vector genome into a packaging cell line for producing a fiber gene-deleted adenovirus vector and providing, wherein said gene for a missing fiber protein is complemented with a gene for a desired modification for targeting the vector to a cell of choice

The invention is further directed to a method for producing a modified adenovirus comprising providing *in vitro* an exogenous fiber protein to a fiberless adenovirus. Additional embodiments of the invention may provide any combination of all of the following steps such that the invention be directed to a method for producing a modified adenovirus comprising: a) providing a packaging cell line for producing a fiberless adenovirus vector, b) introducing into said cell line a helper-independent fiberless or helper-dependent fiberless recombinant adenovirus vector genome, c) growing and harvesting a fiberless adenovirus, d) maintaining the fiberless adenovirus in any suitable buffer, and e) providing exogenous fiber, wherein said fiber may be a modified fiber, to the fiberless adenovirus by adding conditioned media or a soluble fiber preparation or a fiber in any suitable buffer to a virus preparation thereby producing the modified adenovirus.

The invention is further directed to a method for producing a modified adenovirus comprising providing a packaging cell line for producing a helper-dependent fiberless adenovirus vector genome and providing a helper virus vector, wherein said cell line complements at least a deficient fiber protein gene, thereby producing the modified adenovirus. Another aspect of the invention is directed to a method for producing a modified adenovirus comprising: a) providing a packaging cell line for producing a fiberless adenovirus vector, b) introducing into said cell line a helper dependent fiberless recombinant adenovirus vector genome

and a fiberless helper virus vector, c) growing and harvesting a fiberless adenovirus, and d)maintaining the fiberless adenovirus in infectious media, and e) providing exogenous fiber to the fiberless adenovirus by adding conditioned media or a soluble fiber preparation to a virus preparation thereby producing the modified adenovirus.

Additional aspects of the invention are directed to hybrid Ad/AAV vectors and to new helper-dependent vectors used with fiberless adenovirus vectors.

The invention is also directed to a method for delivering a heterologous gene to an EBV-infected B cells comprising infecting said B cells with a pseudotyped Ad5βgal.Δ F particle or other fiber-deleted adenovirus particle, said particle having a chimeric fiber with the receptor-binding knob domain of the adenovirus type 3 fiber.

The invention is also directed to an isolated nucleic acid comprising a post-transcriptional regulatory element (PRE) and a TPL. Preferably the PRE is the woodchuck hepatitis virus PRE (WPRE).

The invention is further directed to a composition for preparing a therapeutic vector, said composition comprising a plasmid comprising an adenovirus genome lacking a nucleotide sequence encoding a fiber protein or a genome that is incapable of expressing sufficient fiber to result in packaging.

Another aspect of the invention is directed to a method of delivering a heterologous gene to a human or any animal comprising providing an exogenous gene to a target cell comprising contacting said cell *in vivo* or *ex vivo* with an amount of a recombinant adenovirus particle sufficient to infect said cell.

The invention is also directed to A method for producing a gutless adenoviral vector particle comprising: a) delivering a helper adenovirus vector genome to an adenovirus vector packaging cell, wherein said helper adenovirus vector genome lacks any gene encoding adenovirus fiber protein or lacks the ability to encode sufficient adenovirus fiber protein to produce an adenoviral vector comprising fiber protein in the absence of complemetation by said packing cell and wherein said packaging cell comprises the nucleic acid molecule of claim 2 operably linked to a promoter and to an adenoviral fiber protein or to a chimeric protein that includes an adenovirus fiber protein tail domain; (b) delivering a gutless adenovirus vector genome to said packaging cell; and (c) recovering the gutless adenoviral vector particle produced by said cell.

Another aspect of the invention is directed to a helper adenovirus particle comprising an adenovirus vector genome that does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-

containing adenovirus particle without complementation of said fiber gene, wherein said genome has a mutation in its packaging sequence that renders said genome substantially incapable of being packaged. Packaging sequence are those sequences are those sequences involved in packaging the viral particle.

The invention is further directed to a helper adenovirus particle comprising an adenovirus vector genome with recombinase sites flanking its packaging sequence, wherein said vector genome does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene.

The invention is also directed to an adenovirus particle comprising a gutless adenoviral vector genome and a fiberless capsid, as well as an adenovirus particle comprising a gutless adenoviral vector genome and a capsid comprising a modified fiber protein.

Another aspect of the invention is directed to a packaging cell for the production of a fiberless or fiber-modified gutless adenovirus particle comprising an adenovirus vector complementing plasmid and a nucleotide sequence encoding a recombinase, wherein said complementing plasmid comprises the nucleic acid molecule of claim 2 operably linked to a promoter and to a nucleotide sequence encoding an adenoviral fiber protein or a chimeric adenoviral fiber protein. Preferably the cell line may comprise a recombinase. In an embodiment of the invention the recombinase may be Cre.

In another embodiment of the invention, the fiber-deleted adenovirus vectors of the invention and the fiber-complementing adenovirus packaging cells of the invention are used to produce a gutless adenovirus vector particle. Such particle comprises a gutless adenoviral vector genome in an adenoviral capsid. The fiber proteins of the capsid may be wild-type fiber, or the modified fiber proteins disclosed herein. Alternatively, such particle may have a fiberless capsid as disclosed herein. Preferably, the gutless genome contains at least one heterologous gene as described herein. As used herein, the term "gutless adenoviral vector genome" means an adenoviral vector genome from which all of the viral genes have been deleted..

The invention also discloses systems or kits for use in any of the aforementioned methods. The systems or kits may contain any appropriate combination of the within-described vectors, plasmids, cell lines, virus particles and additional therapeutic agents as disclosed. Preferably, each such kit or system includes a quantity of the appropriate therapeutic substance or sequence sufficient for at least one administration, and instructions for administration and use. Thus,

one system further comprises an effective amount of a therapeutic agent which enhances the therapeutic effect of the therapeutic viral vector-containing composition. Another variation discloses that the composition and the therapeutic agent are each included in a separate receptacle or container.

It will also be appreciated that any combination of the preceding elements may also be efficacious as described herein, and that all related methods are also within the scope of the present invention.

< Figure 1 is a schematic diagram of the entire adenoviral E4 transcriptional unit with the open reading frames (ORF) indicated by blocked segments along with the promoter and terminator sequences. The location of primers for amplifying specific portions of E4 are also indicated as further described in Example 1A.

Figure 2 is a schematic map of plasmid pE4/Hygro as further described in Example 1B.

Figure 3 is a schematic map of plasmid pCDNA3/Fiber as further described in Example 1B.

Figure 4 is a schematic map of plasmid pCLF as further described in Example 1B.

Figure 5 is a photograph of a Southern blot showing the presence of intact adenovirus E4 3.1 kilobase (kb) insert in the 211 cell line as further described in Example 1C.

Figure 6 is an autoradiograph showing labeled fiber protein immunoprecipitated from cells and electrophoresed under native and denaturing electrophoresis conditions as described in Example 1C. The 293 cells lack fiber while the sublines 211A, 211B and 211R contain fiber protein detectable in functional trimerized form and denatured monomeric form.

Figure 7 is a schematic map of plasmid pDEX/E1 as further described in Example 1D.

Figure 8 is a schematic map of plasmid pE1/Fiber as further described in Example 1F1.

Figure 9 is a schematic map of plasmid pE4/Fiber as further described in Example 1F2).

Figure 10 is a schematic illustration of linearized pD E1Bb gal delivery plasmids for use in cotransfection and recombination to form a recombinant adenoviral vector having multiple adenoviral gene deletions. The plasmids and recombination event are more fully described in Example 2A.

Figure 11 is a schematic of plasmid p11.3 as further described in Example 2A used in the construction of pDV44 delivery plasmid.

Figure 12 is a schematic of plasmid 8.2.

Figure 13 shows the trimeric structure of the recombinant fiber. 293, 211A, 211B, or 211R cells as indicated were metabolically labeled with [^{35}S]methionine, soluble protein extracts prepared, and fiber was immunoprecipitated. A portion of the precipitated protein was electrophoresed on an 8% SDS-PAGE gel under either semi-native or denaturing conditions. The positions of trimeric (T) and monomeric (M) fiber are indicated. As a control for electrophoretic conditions, recombinant Ad2 fiber produced in baculovirus-infected cells was run under identical conditions and stained with Coomassie blue. Figure 14 shows the complementation of a fiber mutant adenovirus by fiber-producing cells. The cell lines indicated ($2x10^6$ cells per sample) were infected with the temperature-sensitive fiber mutant adenovirus H5*ts*142 at 10 PFU/cell and incubated at either the permissive (32.5°C, stippled bars) or the restrictive (39.5°C, solid bars) temperature. 48 hours post-infection, virus was isolated by freeze-thaw lysis and yields determined by fluorescent focus assay on SW480 cells. Each value represents the mean of duplicate samples, and the data shown is representative of multiple experiments.

Figure 15 shows the incorporation of the recombinant Ad5 fiber into Ad3 particles. In Figure 15A, the alignment of the N-terminal (penton base-binding) domains of fiber proteins from several different adenovirus serotypes is shown. From top to bottom, the five different serotypes are listed as SEQ ID NOs 21-25. In Figure 15B, type 3 adenovirus was propagated in 293, 211B, or 211R cells as indicated and purified by two sequential CsCl centrifugations. 10 µg of the purified viral particles was then electrophoresed under denaturing conditions and transferred to a PVDF membrane. Ad5 fiber was detected with a polyclonal rabbit antibody raised against recombinant Ad2 fiber. As a positive control for detection, 400 ng of wild-type Ad2 was run in the lane marked "Ad2". Under these conditions, the mobilities of the Ad2 and Ad5 fibers are indistinguishable and the antibody reacts with both proteins.

Figure 16 shows the fiber deletion in pDV44 and the genomic structures of the Ad5.βgal.ΔF and Ad5.βgal.wt vectors: Figure 16A shows pDV44 that was constructed by removing the fiber gene and residual E3 sequences (nt 30819:32743 of AD5) from pBHG10. Figures 16B shows viruses constructed by cotransfection of either pBHG10 or pDV44 with pΔE1Bβgal. Both are E1/E3 deleted Ad5 vectors, and Ad5.βgal.ΔF has the additional fiber (L5) deletion as in pDV44.

Figure 17 shows the analysis of the viral chromosomes. Figure 17A shows the predicted EcoRI restriction maps of Ad5.βgal.wt and Ad5.βgal.ΔF. The 5.9 kb fragment at the right end of the Ad5.βgal.wt genome is reduced to 4.0 kb by the deletion of fiber sequences in Ad5.βgal.ΔF. Figure 17B shows an ethidium bromide-stained gel of EcoRI-digested viral DNA. Figure 17C shows a Southern blot of the gel as described in Example 2 probed either with labeled fiber or E4 sequences.

Figure 18 shows the analysis of vertex proteins in the viral particles. 293 (non-fiber expressing) or 211B (fiber-expressing) cells were infected with Ad5.βgal.wt ('wt') or with Ad5.βgal.ΔF('ΔF') and the resulting viral particles were purified on CsCl gradients. 10 µg of purified virions was then electrophoresed on 5-16% gradient gels and Western blotted. Proteins were detected with polyclonal anti-fiber or anti-penton base antibodies.

Figure 19 shows the infectivity of Ad particles on THP-1 monocytic cells. Figure 19A shows THP-1 cells that were infected with Ad5.βgal.wt or with fiberless Ad5.βgal.ΔF at 100,000 particles/cell. Forty-eight hours after infection, cells were fixed and stained with X-gal and the fraction of infected cells was determined by light microscopy. Figure 19B shows cells that were infected with 1000 particles per cell of Ad5.βgal.wt or with 100,000 particles/cell of Ad5.βgal.ΔF. As indicated, cells were pretreated with 100 µg/ml of recombinant penton base or with 20 µg/ml of recombinant Ad2 fiber.

Figure 20 shows a schematic of improved fiber-complementing cell lines, 633 and 644 as further described in the Examples.

Figures 21 and 22 illustrates pseudotyping of fiberless particles with fiber proteins and infectivity data as further described in the Examples.

Figure 23 shows the ClaI to BglII fragment of Ad5.

Figure 24 shows the plasmid pGRE5-2/EBV

Figure 25 shows the plasmid pGRE5-E1.

Figure 26 shows the plasmid pSE280-E2 BamHI-SmaI.

Figure 27. The fiber-deleted adenovirus vector Ad5.bgal.DF was grown in cells expressing either no fiber (293; 'Ad5.bgal.DF/0'), the Ad5 fiber (633; 'Ad5.bgal.DF/5F'), or the Ad37 fiber with modifications as described in the text (705; 'Ad5.bgal.DF/37F') and CsCl-purified. 10 µg of the purified particles were electrophoresed and transferred to a nylon membrane. As controls, 10 µg of wild-type Ad37 or the fiber gene-containing vector Ad5.bgal.wt or a sample of purified recombinant Ad37 fiber knob were also run. The blot was probed with polyclonal

antisera against recombinant Ad37 fiber or Ad2 fiber proteins. As a loading control, the same filter was reprobed with an antibody against the Ad2 penton base (the anti-Ad2 sera cross-recognized the very similar Ad5 fiber and Ad5 penton base proteins).

Figure 28 shows PCR analysis for fiber presence.

Figure 29 shows the transduction efficiency for fiberless virus with and without soluble fiber.

Figure 30 shows the transduction efficiency of AD5BgF$^-$ on HDF cell line with the presence of different amounts of 633 conditioned media.

To reduce the frequency of contamination with wild-type adenovirus, it is considered desirable to improve either the viral vector or the cell line to reduce the probability of recombination. For example, an adenovirus from a group with less homology to the group C viruses may be used to engineer recombinant viruses with little propensity for recombination with the Ad5 sequence in 293 cells. Similarly, an epithelial cell line -- *e.g.* the cell line known as 293 -- may be used or further modified according to within-disclosed methods which stably expresses adenovirus proteins or polypeptides from Ad3 and/or proteins or polypeptides from another non-group-C or group C serotype; such a cell line would be useful to support adenovirus-derived viral vectors bearing deletions of regulatory and/or structural genes, irrespective of the serotype from which such a vector was derived.

It is also contemplated that the constructs and methods of the present invention will support the design and engineering of chimeric viral vectors which express amino acid residue sequences derived from two or more Ad serotypes. Thus, unlike methods and constructs available prior to the advent of the present disclosure, this invention allows the greatest possible flexibility in the design and preparation of useful viral vectors and cell lines which support their construction and propagation -- all with a decreased risk of recombining with wild-type Ad to produce potentially-harmful recombinants.

In part, the present invention discloses a simpler, alternative means of reducing the recombination between viral and cellular sequences than those discussed in the art. One such means is to increase the size of the deletion in the recombinant virus and thereby reduce the extent of shared sequences between that

virus and any Ad genes present in a packaging cell line -- *e.g.*, the Ad5 genes in 293 cells, or the various Ad genes in the novel cell lines of the present invention.

Therefore, the present invention makes it feasible to engineer and produce novel viral vectors that are able to package and deliver significantly larger foreign nucleic acid sequences for efficacious use in a variety of therapeutic applications, without endangering the subject to whom they are administered, due to their impaired ability to self-replicate in non-complementing cell lines. Due to the fact that "helper" viruses or plasmids need not be used in conjunction with many of the viral vectors of the present invention, those vectors of the present invention are also simpler to use than those previously described in the art.

In order to provide a clearer understanding of the specification and claims, the following definitions are provided.

Adenoviral Vector or Ad-Derived Vector: Any adenovirus-derived plasmid, genome or virus into which a foreign DNA may be inserted or expressed. This term may also be used interchangeably with "viral vector." This "type" of vector may be utilized to carry nucleotide sequences encoding therapeutic proteins or polypeptides to specific cells or cell types in a subject in need of treatment, as described further herein below.

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. Standard polypeptide nomenclature described in *J. Biol. Chem., 243*:3552-59 (1969) and adopted at 37 C.F.R. § § 1.821 - 1.822 is used.

It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those referred to in 37 C.F.R. § § 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as NH_2 or to a carboxyl-terminal group such as COOH.

Complementing Plasmid: This term is generally used herein to describe plasmid vectors used to deliver particular nucleotide sequences into a packaging cell line, with the intent of having said sequences stably integrate into the cellular genome.

Delivery Plasmid: This term is generally used herein to describe a plasmid vector that carries or delivers nucleotide sequences in or into a cell line (*e.g.*, a packaging cell line) for the purpose of propagating therapeutic viral vectors of the present invention.

DNA Homolog: A nucleic acid having a preselected conserved nucleotide sequence and a sequence encoding a preferred polypeptide according to the present invention, where the nucleic acid is substantial homologous to a named preferred embodiment. By the term "substantially homologous" is meant having at least 80%, preferably at least 90%, most preferably at least 95% homology therewith.

The terms "homology" and "identity" are often used interchangeably. In this regard, percent homology or identity may be determined, for example, by comparing sequence information using a GAP computer program. The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443 (1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482 (1981). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program may include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745 (1986), as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Whether any two nucleic acid molecules have nucleotide sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA 85*:2444 (1988). Alternatively the BLAST function of the National Center for Biotechnology Information database may be used to determine identity

In general, sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated

using published techniques. (See, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988). Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al., Nucleic Acids Research 12(I)*:387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al., J Molec Biol 215*:403 (1990)).

Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide. For example, a test polypeptide may be defined as any polypeptide that is 90% or more identical to a reference polypeptide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polynucleotide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons may be made between a test and reference polynucleotides. Such differences may be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they may be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions.

An embodiment of the invention may use polynucleotides at least 90% or 95% identical to those encoding the TPL nucleic acid sequences. A further embodiment of the invention may include those polynucleotides that encode a

polypeptide of interest that are at least 95% identical when the variation in such a polynucleotide is due to more than merely degenerate changes.

Expression or Delivery Vector: Any plasmid or virus into which a foreign DNA may be inserted for expression in a suitable host cell -- *i.e.*, the protein or polypeptide encoded by the DNA is synthesized in the host cell's system. Vectors capable of directing the expression of DNA segments (genes) encoding one or more proteins are referred to herein as "expression vectors." Also included are vectors which allow cloning of cDNA (complementary DNA) from mRNAs produced using reverse transcriptase.

Foreign Gene: This term is used to identify a DNA molecule not present in the exact orientation and position as the counterpart DNA molecule found in wild-type adenovirus. It may also refer to a DNA molecule from another organism or species (*i.e.*, exogenous) or from another Ad serotype.

Gene: A nucleic acid whose nucleotide sequence encodes an RNA or polypeptide. A gene can be either RNA or DNA. Genes may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Isolated: This term is used to indicate a nucleic acid or polypeptide sequence separated from the genetic environment from which the sequences were obtained. It may also mean altered from the natural state. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Thus, a polypeptide or polynucleotide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" or an "isolated polynucleotide" are polypeptides or polynucleotides that have been purified, partially or substantially, from a recombinant host cell or from a native source. For example, a recombinantly produced version of a compounds can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988). The terms isolated and purified are sometimes used interchangeably.

By "isolated" is meant that the DNA is free of the coding sequences of those genes that, in the naturally-occurring genome of the organism (if any) from which the DNA of the invention is derived, immediately flank the gene encoding the DNA of the invention. The isolated DNA may be single-stranded or double-stranded, and may be genomic DNA, cDNA, recombinant hybrid DNA, or synthetic DNA. It

may be identical to a native DNA sequence, or may differ from such sequence by the deletion, addition, or substitution of one or more nucleotides.

Isolated or purified as it refers to preparations made from biological cells or hosts should be understood to mean any cell extract containing the indicated DNA or protein including a crude extract of the DNA or protein of interest. For example, in the case of a protein, a purified preparation can be obtained following an individual technique or a series of preparative or biochemical techniques and the DNA or protein of interest can be present at various degrees of purity in these preparations. The procedures may include for example, but are not limited to, ammonium sulfate fractionation, gel filtration, ion exchange change chromatography, affinity chromatography, density gradient centrifugation and electrophoresis.

A preparation of DNA or protein that is "pure" or "isolated" should be understood to mean a preparation free from naturally occurring materials with which such DNA or protein is normally associated in nature. "Essentially pure" should be understood to mean a "highly" purified preparation that contains at least 95% of the DNA or protein of interest.

A cell extract that contains the DNA or protein of interest should be understood to mean a homogenate preparation or cell-free preparation obtained from cells that express the protein or contain the DNA of interest. The term "cell extract" is intended to include culture media, especially spent culture media from which the cells have been removed.

Packaging Cell line: A packaging cell line is a cell line that provides a missing gene product or its equivalent.

Particle: The adenovirus (Ad) particle is relatively complex and may be resolved into various substructures. The particle is the minimal structural or functional unit of a virus. A virus can refer to a single particle, a stock of particles or a viral genome.

Penton: The terms "penton" or "penton complex" are preferentially used herein to designate a complex of penton base and fiber. The term "penton" may also be used to indicate penton base, as well as penton complex. The meaning of the term "penton" alone should be clear from the context within which it is used.

Plasmid: An autonomous self-replicating extrachromosomal circular DNA

Post-transcriptional Regulatory Element (PRE) is a regulatory element found in viral or cellular messenger RNA that is not spliced, i.e. intronless messages. Examples include, but are not limited to, human hepatitis virus, woodchuck

hepatitis virus, the TK gene and mouse histone gene. The PRE may be placed before a polyA sequence and after a heterologous DNA sequence.

Pseudotyping: This term as generally used herein describes the production of adenoviral vectors having modified capsid protein or capsid proteins from a different serotype than the serotype of the vector itself. One example, is the production of an adenovirus 5 vector particle containing a chimeric Ad3/Ad5 fiber protein. This may be accomplished by producing the adenoviral vector in packaging cell lines expressing different fiber proteins.

Promoter: Useful promoters according to the present invention may be inducible or constitutive. Inducible promoters will initiate transcription only in the presence of an additional molecule; constitutive promoters, on the other hand, do not require the presence of any additional molecule to regulate gene expression. A regulatable or inducible promoter may also be described as a promoter wherein the rate of RNA polymerase binding and initiation is modulated by external stimuli. Such stimuli include various compounds or compositions, light, heat, stress, chemical energy sources, and the like. Inducible, suppressible and repressible promoters are considered regulatable promoters.

Receptor: Receptor is a term used herein to indicate a biologically active molecule that specifically binds to (or with) other molecules. The term "receptor protein" may be used to more specifically indicate the proteinaceous nature of a specific receptor.

Recombinant: As used herein, the term is intended to refer to any progeny formed as the result of genetic engineering. This may also be used to describe a virus formed by recombination of plasmids in a packaging cell.

Transgene or Therapeutic Nucleotide Sequence: As described and claimed herein, such a sequence includes DNA and RNA sequences encoding an RNA or polypeptide. Such sequences may be "native" or naturally-derived sequences; they may also be "non-native" or "foreign" sequences which are naturally- or recombinantly-derived. The term "transgene," which may be used interchangeably herein with the term "therapeutic nucleotide sequence," is often used to describe a heterologous or foreign (exogenous) gene that is carried by a viral vector and transduced into a host cell.

Therefore, therapeutic nucleotide sequences may also include antisense sequences or nucleotide sequences which may be transcribed into antisense sequences. Therapeutic nucleotide sequences (or transgenes) further comprise sequences which function to produce a desired effect in the cell or cell nucleus into which said therapeutic sequences are delivered. For example, a therapeutic

nucleotide sequence may encode a functional protein intended for delivery into a cell which is unable to produce that functional protein.

Adenovirus

Fiber plays a crucial role in adenovirus infection by attaching the virus to a specific receptor on the cell surface. The fiber is an elongated protein which exists as a trimer of three identical polypeptides (polypeptide IV) of 582 amino acids in length. An adenovirus fiber consists of three domains: an N-terminal tail domain that interacts with penton base; a shaft composed of variable numbers of repeats of a 15-amino-acid segment that forms beta-sheet and beta-bends; and a knob at the C-terminus ("head domain") that contains the type-specific antigen and is responsible for binding to the cell surface receptor. The gene encoding the fiber protein from Ad2 has been expressed in human cells and has been shown to be correctly assembled into trimers, glycosylated and transported to the nucleus. (See, *e.g.*, Hong and Engler, *Virology 185*: 758-761, 1991). Thus, alteration of the fiber in recombinant Ad vectors can lead to alteration in gene delivery. This has great utility for a variety of gene therapy applications and is one of the objects of the present invention.

Hexon, penton and fiber capsomeres are the major components on the surface of the virion. Their constituent polypeptides, nos. II, III and IV, contain tyrosine residues that are exposed on the surface of the virion and can be labeled -- *e.g.*, by iodination of intact particles.

The 35,000+ base pair (bp) genome of adenovirus type 2 has been sequenced and the predicted amino acid sequences of the major coat proteins (hexon, fiber and penton base) have been described. (See, *e.g.*, Neumann *et al., Gene 69*: 153-157 (1988); Herisse *et al., Nuc. Acids Res. 9*: 4023-4041 (1981); Roberts *et al., J. Biol. Chem. 259*: 13968-13975 (1984); Kinloch *et al., J. Biol. Chem. 259*: 6431-6436 (1984); and Chroboczek *et al., Virol. 161*: 549-554, 1987).

The sequence of Ad5 DNA was completed more recently; its sequence includes a total of 35,935 bp. Portions of many other adenovirus genomes have also been sequenced. It is presently understood that the upper packaging limit for adenovirus virions is about 105% of the wild-type genome length. (See, *e.g.*, Bett, *et al., J. Virol. 67(10):* 5911-21, 1993). Thus, for Ad2 and Ad5, this would be an upper packaging limit of about 38kb of DNA.

Adenovirus DNA also includes inverted terminal repeat sequences (ITRs) ranging in size from about 100 to 150 bp, depending on the serotype. The inverted repeats enable single strands of viral DNA to circularize by base-pairing

of their terminal sequences, and the resulting base-paired "panhandle" structures required for replication of the viral DNA.

For efficient packaging, the ITRs and the packaging signal (a few hundred bp in length) comprise the "minimum requirement" for replication and packaging of a genomic nucleic acid into an adenovirus particle. Helper-dependent vectors lacking all viral ORFs but including these essential *cis* elements (the ITRs and contiguous packaging sequence) have been constructed, but the virions package less efficiently that the helper and package as multimers part of the time, which suggests that the virus may "want" to package a fuller DNA complement (see, *e.g.*, Fisher, *et al., Virology 217*: 11-22, 1996).

The viral vectors of the present invention may retain their ability to express the genome packaged within -- *i.e.*, they may retain their "infectivity" -- they do not act as infectious agents, however, to the extent that they cause disease in the subjects to whom they are administered for therapeutic purposes.

It is to be appreciated that Ad vectors have several distinct advantages over other viral vectors in the art. For example, recombination of such vectors is rare; there are no known associations of human malignancies with adenoviral infections despite common human infection with adenoviruses; the genome may be manipulated to accommodate foreign genes of a fairly substantial size; and host proliferation is not required for expression of adenoviral proteins.

An extension of this invention is that the Ad-derived viral vectors disclosed herein may be used to target and deliver genes into specific cells by incorporating the attachment sequence for other receptors (such as CD4) onto the fiber protein by recombinant DNA techniques, thus producing a chimeric molecule. This should result in the ability to target and deliver genes into a wide range of cell types with the advantage of evading recognition by the host's immune system. The within-disclosed delivery systems thus provide for increased flexibility in gene design to enable gene delivery into proliferating and nonproliferating cell types.

For example, U.S. Patent Nos. 5,756,086 and 5,543,328 as well as, WO95/26412 and WO 98/44121 and Krasnykh, *et al.* (*J. Virol. 70:* 6839-46, 1996) describe modifications that may be made to the adenovirus fiber protein. Such modifications are useful in altering the targeting mechanism and specificity of adenovirus and could readily be utilized in conjunction with the constructs of the present invention to target the novel viral vectors disclosed herein to different receptors and different cells. Moreover, modifications to fiber protein which alter its tropism may permit greater control over the localization of viral vectors in therapeutic applications.

Similarly, incorporation of various structural proteins into cell lines of the present invention, whether or not those proteins are modified, is also contemplated by the present invention. Thus, for example, modified penton base polypeptides such as those described in Wickham, *et al.* (*J. Virol. 70*: 6831-8, 1996) may have therapeutic utility when used according to the within-disclosed methods.

While some of the Examples appearing below specifically recite fiber proteins, polypeptides, and fragments thereof, it is expressly provided herein that other structural and non-structural Ad proteins and polypeptides (*e.g.*, regulatory proteins and polypeptides) may be used as components of the various disclosed vectors and cell lines. Moreover, chimeric molecules comprised of proteins, polypeptides, and/or fragments thereof which are derived from different Ad serotypes may be used in any of the within-disclosed methods, constructs and compositions. Similarly, recombinant DNA sequences of the present invention may be prepared using nucleic acid sequences derived from different Ad serotypes, in order to design useful constructs with broad applicability, as disclosed and claimed herein.

It should also be appreciated that, while the members of Group C or Group D adenovirus – *i.e.*, Ad serotypes 1, 2, 5, 6 or 37 -- are specifically recited in various examples herein, the present invention is in no way limited to those serotypes alone. In view of the fact that the adenovirus serotypes are all closely-related in structure and functionality, therapeutic viral vectors, packaging cell lines, and plasmids of the present invention may be constructed from components of any and all Ad serotypes -- and the within-disclosed methods of making and using the various constructs and cell lines of the present invention apply to all of said serotypes.

The family of Adenoviridae includes many members with at least 47 known serotypes of human adenovirus (Ad1-Ad47) (Shenk, *Virology*, Chapter 67, *in* Fields *et al.*, eds. Lippincott-Raven, Philadelphia, 1996,) as well as members of the genus Mastadenovirus including human, simian, bovine, equine, porcine, ovine, canine and opossum viruses, and members of the Aviadenovirus genus, including bird viruses, e.g. CELO. Thus it is contemplated that the disclosed inventions can be applied to any adenovirus species, and the invention need not be so limited. One of skill in the art would have knowledge of the different adenoviruses as evidenced by (Shenk, *Virology*, Chapter 67, *in* Fields *et al.*, eds. Lippincott-Raven, Philadelphia, 1996,) which is herein incorporated by reference.

Packaging Cell Lines A. Adenovirus Complementation Genetics

The first generation of recombinant adenoviral vectors currently available typically have a deletion in the first viral early gene region which is generally referred to as E1, which comprises the E1a and E1b regions. (These regions span genetic map units 1.30 to 9.24.) Figure 3 in chapter 67 of *Fields Virology*, 3d Ed. (Fields *et al.* eds, Lippincott-Raven Publ., Philadelphia, 1996, p. 2116) illustrates a transcription and translation map of adenovirus type 2 (Ad2).

Deletion of the viral E1 region renders the recombinant adenovirus defective for replication and incapable of producing infectious viral particles in subsequently-infected target cells. Thus, to generate E1-deleted adenovirus genome replication and to produce virus particles requires a system of complementation which provides the missing E1 gene product. E1 complementation is typically provided by a cell line expressing E1, such as the human embryonic kidney packaging cell line, i.e. an epithelial cell line, called 293. Cell line 293 contains the E1 region of adenovirus, which provides E1 gene region products to "support" the growth of E1-deleted virus in the cell line (see, *e.g.*, Graham *et al., J. Gen. Virol. 36*: 59-71, 1977). Additionally, cell lines that may be usable for production of defective adenovirus having a portion of the adenovirus E4 region have been reported (WO 96/22378). Multiply deficient adenoviral vectors and complementing cell lines have also been described (WO 95/34671, U.S. Patent No. 5,994,106). Nevertheless, inherent problems exist concerning first-generation recombinant adenoviruses.

B. Adenovirus Particle Packaging Cell Lines

Packaging cell lines disclosed herein support viral vectors with deletions of major portions of the viral genome, without the need for helper viruses. Additionally, the invention provides novel cell lines and helper viruses for use with helper-dependent vectors.

Thus, in one embodiment of the present invention, a packaging cell line is disclosed having DNA sequences stably integrated into the cellular genome wherein the DNA sequences encode one or more adenovirus regulatory and/or structural polypeptides which complement the genes deleted or mutated in the adenovirus vector genome to be replicated and packaged.

In another embodiment, the packaging cell line expresses one or more adenovirus structural proteins, polypeptides, or fragments thereof, wherein said

structural protein is selected from the group consisting of penton base, hexon, fiber, polypeptide IIIa, polypeptide V, polypeptide VI, polypeptide VII, polypeptide VIII, and biologically active fragments thereof.

In one variation, the sequences are constitutively expressed; in another, one or more sequences is under the control of a regulatable promoter. In a preferred embodiment expression is constitutive. In various preferred embodiments, the polypeptides expressed by the DNA sequences are biologically active.

In a further and preferred embodiment the packaging cell line of the present invention supports the production of a viral vector. In a preferred embodiment the viral vector is a therapeutic vector.

The present invention also discloses a packaging cell line which complements a viral vector having a deletion or mutation of a DNA sequence encoding an adenovirus structural protein, regulatory polypeptides E1A and E1B, and/or one or more of the following regulatory proteins or polypeptides: E2A, E2B, E3, E4, L4, or fragments thereof.

Various useful packaging cells are contemplated which complement adenovirus. In one aspect of the present invention, each DNA sequence is introduced into the genome of the within-disclosed cell lines via a separate complementing plasmid. In other embodiments, two or more DNA sequences were introduced into the genome via a single complementing plasmid. In one variation, the complementing plasmid comprises a DNA sequence encoding adenovirus fiber protein, polypeptide or fragment thereof. An example of a useful complementing plasmid according to the present invention is a plasmid having the characteristics of pCLF (for deposit details, see Example 3)

One embodiment discloses a packaging cell useful in the preparation of recombinant adenovirus viral vectors comprising a delivery plasmid comprising an adenovirus genome lacking a nucleotide sequence encoding fiber. In one variation, the delivery plasmid further comprises a nucleotide sequence encoding a foreign polypeptide. A preferred delivery plasmid is pDV44, pE1B gal, or pE1sp1B. In another variation, the cell further comprises a complementing plasmid containing a nucleotide sequence encoding fiber, the plasmid being stably integrated into the cellular genome of the cell.

In one embodiment, a composition comprises a cell containing first and second delivery plasmids wherein a first delivery plasmid comprises an adenovirus genome lacking a nucleotide sequence encoding fiber and incapable of directing the packaging of new viral particles in the absence of a second

delivery plasmid, and a second delivery plasmid comprises an adenoviral genome capable of directing the packaging of new viral particles in the presence of the first delivery plasmid.

In another variation, the first and second delivery plasmids interact within the cell to produce a therapeutic viral vector. In yet another variation, the cell further comprises a complementing plasmid containing a nucleotide sequence encoding fiber, the plasmid being stably integrated into the cellular genome of the cell. In still another, the first or second delivery plasmid further comprises a nucleotide sequence encoding a foreign polypeptide. In various embodiments, the polypeptide is a therapeutic molecule.

Another embodiment discloses a composition as before, wherein the first delivery plasmid lacks adenovirus packaging signal sequences. In another aspect, the second delivery plasmid contains a LacZ reporter construct. In another variation, the second delivery plasmid further lacks a nucleotide sequence encoding an adenovirus regulatory protein. In one variation, the regulatory protein is E1. In one embodiment of the above-noted compositions, the complementing plasmid has the characteristics of pCLF.

In another embodiment, a composition is disclosed wherein the first delivery plasmid lacks a nucleotide sequence encoding an adenovirus structural protein and the second delivery plasmid lacks a nucleotide sequence encoding adenovirus E1 protein. In another, the first delivery plasmid lacks a nucleotide sequence encoding adenovirus E4 protein and the second delivery plasmid lacks a nucleotide sequence encoding adenovirus E1 protein. In still another, the cell contains at least one complementing plasmid encoding an adenoviral regulatory protein and a structural protein.

In one preferred variation of the present invention, a packaging cell line expresses fiber protein. In one embodiment, the fiber protein has been modified to include a non-native amino acid residue sequence which targets a specific receptor, but which does not disrupt trimer formation or transport of fiber into the nucleus. In another variation, the non-native amino acid residue sequence alters the binding specificity of the fiber for a targeted cell type. In still another embodiment, the structural protein is fiber comprising amino acid residue sequences from more than one adenovirus serotype. As disclosed herein, the nucleotide sequences encoding fiber protein or polypeptide need not be modified solely at one or both termini; fiber protein -- and indeed, any of the adenovirus structural proteins, as taught herein -- may be modified "internally" as well as at the termini.

In one variation, the non-native amino acid residue sequence is coupled to the carboxyl terminus of the fiber. In yet another, the non-native amino acid residue sequence further includes a linker sequence. Alternatively, the fiber protein further comprises a ligand coupled to the linker. A suitable ligand may be selected from the group consisting of ligands that specifically bind to a cell surface receptor and ligands that can be used to couple other proteins or nucleic acid molecules. Typically, any of the packaging cell lines of this invention may have a DNA sequence encoding all or part of a fiber protein -- including modified or chimeric proteins -- stably integrated into the genome.

In various aspects of the present invention, a packaging cell line of the present invention is derived from a procaryotic cell line; in another, it is derived from a eucaryotic cell line. While various embodiments suggest the use of mammalian cells, and more particularly, epithelial cell lines, a variety of other, non-epithelial cell lines are used in various embodiments. Thus, while various embodiments disclose the use of a cell line selected from the group consisting of 293, A549, W162, HeLa, Vero, 211, and 211A cell lines, it is understood that various other cell lines are likewise contemplated for use as disclosed herein.

Therapeutic Viral Vectors and Related Systems

A. Nucleic Acid Segments

A therapeutic viral vector or composition of the present invention comprises a nucleotide sequence, nucleic acid molecule or segment as described herein. Typically, the nucleic acid molecule or molecule encodes a protein or polypeptide molecule -- or a biologically active fragment thereof -- which may be used for therapeutic applications. A nucleotide sequence may further comprise an enhancer element or a promoter located 3' or 5' to and controlling the expression of such a therapeutic nucleotide sequence or gene.

A subject nucleotide sequence consists of a nucleic acid molecule that comprises at least 2 different operatively linked DNA segments. The DNA can be manipulated and amplified by PCR as described herein and by using standard techniques, such as those described in *Molecular Cloning: A Laboratory Manual, 2nd Ed.*, Sambrook *et al.*, eds., Cold Spring Harbor, New York (1989). Typically, to produce a nucleotide sequence of the present invention, the sequence encoding the selected polypeptide and the promoter or enhancer are operatively linked to a DNA molecule capable of autonomous replication in a cell either *in vivo* or *in vitro*.

By operatively linking the enhancer element or promoter and the nucleotide sequence to the vector, the attached segments are replicated along with the vector sequences.

Thus, a recombinant DNA molecule (rDNA) of the present invention is a hybrid DNA molecule comprising at least 2 nucleotide sequences not normally found together in nature. In various preferred embodiments, one of the sequences is a sequence encoding an Ad-derived polypeptide, protein, or fragment thereof. Stated another way, a nucleotide sequence of the present invention is one that encodes an expressible protein, polypeptide or fragment thereof, and it may further include an active constitutive or regulatable (*e.g.* inducible) promoter sequence.

A nucleotide sequence of the present invention is optimally from about 20 base pairs to about 40,000 base pairs in length. Preferably the nucleotide sequence is from about 50 bp to about 38,000 bp in length. In various embodiments, the nucleotide sequence is of sufficient length to encode one or more adenovirus proteins or functional polypeptide portions thereof. Since individual Ad polypeptides vary in length from about 19 amino acid residues to about 967 amino acid residues, corresponding nucleotide sequences will range from about 50 bp up to about 3000 bp, depending on the number and size of individual polypeptide-encoding sequences that are "replaced" in the viral vectors by therapeutic nucleotide sequences of the present invention.

1. Tripartite Leader (TPL) Nucleic Acid Sequences

In one aspect of the invention, it has been discovered that expression of adenovirus late proteins such as the structural proteins in a packaging cell line according to the present invention is substantially improved when the expression cassette present on the complementing plasmid or in the packaging cell line's genome contains an adenovirus tripartite leader (TPL) nucleic acid sequence.

Thus, the invention contemplates a nucleic acid molecule comprising a TPL nucleotide sequence. Preferably, the TPL nucleotide sequence may be operatively linked to an intron containing RNA processing signals (such as for example, splice donor or splice acceptor sites) suitable for expression in the packaging cell line. Most preferably, the intron contains a splice donor site and a splice acceptor site. Alternatively, the TPL nucleotide sequence may not comprise an intron.

In one embodiment, a subject nucleic acid molecule of this invention is isolated, *i.e.*, separated from the genetic environment from which the component sequences were obtained. Thus, molecular cloning of fragments of a gene will

produce an isolated nucleic acid, as will the chemical synthesis of an oligonucleotide to build a nucleic acid molecule.

The intron useful in the present invention is any nucleotide sequence which functions in the packaging cell line to provide RNA processing signals, as are well known in the art, including splicing signals. Introns have been well characterized from a large number of structural genes, and therefor the invention should not be considered as limited. Well characterized and preferred introns include a native intron 1 from adenovirus, such as Ad5's TPL intron 1; others include the SV40 VP intron; the rabbit beta-globin intron, and synthetic intron constructs. See, for example, Petitclerc *et al., J. Biothechnol., 40*:169, 1995, and Choi *et al., Mol. Cell. Biol., 11*:3070, 1991.

The TPL nucleotide sequence comprises either (a) first and second TPL exons or (b) first, second and third TPL exons, where each TPL exon in the sequence is selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3. A complete exon is one which contains the complete nucleic acid sequence based on the sequence found in the wild type viral genome. Preferably the TPL exons are from Ad2, Ad3, Ad5, Ad7 and the like, however, they may come from any Ad serotype, as described herein. A preferred partial TPL exon 1 is described in the Examples. The use of a TPL with a partial exon 1 has been reported (WO98/13499).

The intron and the TPL exons can be operatively linked in a variety of configurations to provide a functional TPL nucleotide sequence, although in some embodiments of the invention, an intron may not be a part of the construct. For example, the intron can be positioned between any of TPL exons 1, 2 or 3, and the exons can be in any order of first and second, or first/second/third. The intron can also be placed preceding the first TPL exon or following the last TPL exon. In a preferred embodiment, complete TPL exon 1 is operatively linked to complete TPL exon 2 operatively linked to complete TPL exon 3. In a preferred variation, adenovirus TPL intron 1 is positioned between complete TPL exon 1 and complete TPL exon 2. It may also be possible to use analogous translational regulators from other viral systems such as rabiesvirus.

A preferred "complete" TPL nucleic acid molecule containing complete TPL exons 1, 2 and 3 with adenovirus intron 1 inserted between exons 1 and 2 has a nucleotide sequence shown in SEQ ID NO: 32. A preferred "partial" TPL nucleic acid molecule containing partial TPL exon 1 and complete TPL exons 2 and 3 in that order has a nucleotide sequence shown in SEQ ID NO: 26. The construction of these preferred TPL nucleotide sequences is described in the Examples.

Thus, preferred expression cassettes and complementing plasmids for expressing adenovirus structural genes, particularly fiber protein, contain an adenovirus TPL nucleotide sequence as described herein. Preferred packaging cell lines containing the subject nucleic acid molecules also contain a TPL nucleotide sequence of the invention.

2. Complementing Plasmids

The invention describes in a related embodiment nucleic acid molecules and nucleotide sequences, typically in the form of DNA plasmid vectors, which are capable of expression of an adenovirus structural protein or regulatory protein. Because these expression plasmids are used to complement the defective genes of a recombinant adenovirus vector genome of this invention, the plasmids are referred to as complementing or complementation plasmids.

The complementing plasmid contains an expression cassette, a nucleotide sequence capable of expressing a protein product off the gene encoded by the nucleotide sequence. Expression cassettes are described in more detail herein, but typically contain a promoter and a structural gene operatively linked to the promoter and whose expression is controlled by the promoter. A preferred complementing plasmid further includes a TPL nucleotide sequence described herein to enhance expression of the structural gene product when used in the context of adenovirus genome replication and packaging.

In one embodiment, a complementing plasmid comprises a promoter nucleotide sequence operatively linked to a nucleotide sequence encoding an adenovirus structural polypeptide. The adenovirus structural polypeptide is selected from the group consisting of penton base; hexon; fiber; polypeptide IIIa; polypeptide V; polypeptide VI; polypeptide VII; polypeptide VIII; and biologically active fragments thereof. In another variation, a complementing plasmid further comprises a nucleotide sequence encoding a first adenovirus regulatory polypeptide, a nucleotide sequence encoding a second regulatory polypeptide, a nucleotide sequence encoding a third regulatory polypeptide; or any combination of the foregoing.

The present invention also discloses a complementing plasmid comprising a promoter nucleotide sequence operatively linked to a nucleotide sequence encoding an adenovirus structural protein, polypeptide or fragment thereof and a nucleotide sequence encoding an adenovirus regulatory protein, polypeptide or fragment thereof. In one variation, the early region polypeptide is E4; in another,

the plasmid comprises pE4/Hygro. In still another variation, the early region polypeptides are E1 and E4.

In another aspect of the present invention, the complementing plasmid used to transform a cell line of the present invention further comprises a DNA sequence encoding an adenovirus regulatory protein, polypeptide or fragment thereof. In one variation, the regulatory protein is selected from the group consisting of E1A, E1B, E2A, E2B, E3, E4 and L4 (also referred to as "the 100K protein"); an exemplary complementing plasmid has the characteristics of is pE4/Hygro (for deposit details, see the Examples). In another aspect, the complementing plasmid used to transform a cell line of the present invention further comprises a DNA sequence encoding two or more of the above mentioned adenovirus regulatory proteins, polypeptides or fragments thereof.

Preferred complementing plasmids include pCLF, pDV60, pDV61, pDV67, pDV69, pDV80, pDV90 and the like plasmids described in the Examples. The nucleic acid sequence of particularly preferred complementing plasmids are shown in SEQ ID NO: 43 for pDV60, SEQ ID NO: 44 for pDV67, SEQ ID NO: 47 for pDV69, SEQ ID NO: 64 for pDV80 and SEQ ID NO: 65 for pDV90.

3. Nucleic Acid Molecule Synthesis

A nucleic acid molecule comprising synthetic oligonucleotide sequences of the present invention can be prepared using any suitable method, such as, the phosphotriester or phosphodiester methods. See Narang *et al., Meth. Enzymol.*, 68:90, (1979); U.S. Patent No. 4,356,270; and Brown *et al., Meth. Enzymol.*, 68:109, (1979).

For oligonucleotide sequences in which a family of variants is preferred, the synthesis of the family members can be conducted simultaneously in a single reaction vessel, or can be synthesized independently and later admixed in preselected molar ratios. For simultaneous synthesis, the nucleotide residues that are conserved at preselected positions of the sequence of the family member can be introduced in a chemical synthesis protocol simultaneously to the variants by the addition of a single preselected nucleotide precursor to the solid phase oligonucleotide reaction admixture when that position number of the oligonucleotide is being chemically added to the growing oligonucleotide polymer. The addition of nucleotide residues to those positions in the sequence that vary can be introduced simultaneously by the addition of amounts, preferably equimolar amounts, of multiple preselected nucleotide precursors to the solid phase

oligonucleotide reaction admixture during chemical synthesis. For example, where all four possible natural nucleotides (A,T,G and C) are to be added at a preselected position, their precursors are added to the oligonucleotide synthesis reaction at that step to simultaneously form four variants.

This manner of simultaneous synthesis of a family of related oligonucleotides has been previously described for the preparation of "Degenerate Oligonucleotides" by Ausubel *et al. (Current Protocols in Molecular Biology,* Suppl. 8. p.2.11.7, John Wiley & Sons, Inc., New York ,1991), and can readily be applied to the preparation of the therapeutic oligonucleotide compositions described herein.

Nucleotide bases other than the common four nucleotides (A,T,G or C), or the RNA equivalent nucleotide uracil (U), can also be used in the present invention. For example, it is well known that inosine (I) is capable of hybridizing with A, T and G, but not C. Examples of other useful nucleotide analogs are known in the art and may be found referred to in 37 C.F.R. §1.822.

Thus, where all four common nucleotides are to occupy a single position of a family of oligonucleotides, that is, where the preselected nucleotide sequence is designed to contain oligonucleotides that can hybridize to four sequences that vary at one position, several different oligonucleotide structures are contemplated. The composition can contain four members, where a preselected position contains A,T,G or C. Alternatively, a composition can contain two nucleotide sequence members, where a preselected position contains I or C, and has the capacity the hybridize at that position to all four possible common nucleotides. Finally, other nucleotides may be included at the preselected position that have the capacity to hybridize in a non-destabilizing manner with more than one of the common nucleotides in a manner similar to inosine.

Similarly, larger nucleic acid molecules can be constructed in synthetic oligonucleotide pieces, and assembled by complementary hybridization and ligation, as is well known.

B. Adenovirus Expression Vector Systems

One key component of the present invention for producing gene therapy reagents comprised of recombinant adenovirus particles is the recombinant adenovirus vector genome which is encapsulated in the virus particle and which expresses exogenous genes in a gene therapy setting. Thus, the components of an recombinant adenovirus vector genome include the ability to express selected

adenovirus structural genes, to express a desired exogenous protein, and to contain sufficient replication and packaging signals that the genome is packaged into a gene delivery vector particle. The preferred replication signal is an adenovirus inverted terminal repeat containing an adenovirus origin of replication, as is well known and described herein.

According to the present invention, a preferred recombinant adenovirus vector genome is "helper independent" which means the genome can replicate and be packaged without the help of a second, complementing helper virus. Instead, the complementation is provided by a packaging cell line of the present invention. Additional embodiments of the invention, however, are drawn to a vector genome referred to as "gutless" which is "helper dependent."

In a preferred embodiment, the adenovirus vector genome does not encode a functional adenovirus fiber protein. A non-functional fiber gene refers to a deletion, mutation or other modification to the adenovirus fiber gene such that the gene does not express any or insufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of the fiber gene by a complementing plasmid or packaging cell line. Such a genome is referred to as a "fiberless" genome, not to be confused with a fiberless particle. Alternatively, a fiber protein may be encoded but is insufficiently expressed to result in a fiber containing particle.

Thus, the invention describes a helper-independent fiberless recombinant adenovirus vector genome comprising genes which (a) express all adenovirus structural gene products but express insufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene, (b) express an exogenous protein, and (c) contains an adenovirus packaging signal and inverted terminal repeats containing adenovirus origin of replication.

The introduction of exogenous DNA into eucaryotic cells has become one of the most powerful tools of the molecular biologist. The term "exogenous" encompasses any therapeutic composition of this invention which is administered by the therapeutic methods of this invention. Thus, "exogenous" may also be referred to herein as "foreign," "non-native," and the like. The methods of this invention preferably require efficient delivery of the DNA into the nucleus of the recipient cell and subsequent identification of cells that are expressing the foreign DNA.

The adenovirus vector genome is propagated in the laboratory in the form of rDNA plasmids containing the genome, and upon introduction into an appropriate

host, the viral genetic elements provide for viral genome replication and packaging rather than plasmid-based propagation. Exemplary methods for preparing an Ad-vector genome are described in the Examples.

A widely-used plasmid is pBR322, a vector whose nucleotide sequence and endonuclease cleavage sites are well known. Various other useful plasmid vectors are described in the Examples that follow.

A nucleic acid vector of the present invention comprises a nucleic acid (preferably DNA) molecule capable of autonomous replication in a cell and to which a DNA segment, *e.g.*, a gene or polynucleotide, can be operatively linked so as to bring about replication of the attached segment. In the present invention, one of the nucleotide segments to be operatively linked to vector sequences encodes at least a portion of a therapeutic nucleic acid molecule -- in effect, a nucleic acid sequence that encodes one or more therapeutic proteins or polypeptides, or fragments thereof.

As one of skill in the art will note, in various embodiments of the present invention, different "types" of vectors are disclosed. For example, one "type" of vector is used to deliver particular nucleotide sequences into a packaging cell line, with the intent of having said sequences stably integrate into the cellular genome; these "types" of vectors are generally identified herein as complementing plasmids. A further "type" of vector described herein carries or delivers nucleotide sequences in or into a cell line (*e.g.*, a packaging cell line) for the purpose of propagating therapeutic viral vectors of the present invention; hence, these vectors are generally referred to herein as delivery plasmids. A third "type" of vector described herein is utilized to carry nucleotide sequences encoding therapeutic proteins or polypeptides to specific cells or cell types in a subject in need of treatment; these vectors are generally identified herein as therapeutic viral vectors or Ad-derived vectors and are in the form of a virus particle encapsulating a viral nucleic acid containing an expression cassette nucleic acid sequence for expressing the therapeutic gene.

1. Nucleic Acid Gene Expression Cassettes

In various embodiments, a peptide-coding sequence of the therapeutic gene is inserted into an expression vector and expressed; however, it is also feasible to construct an expression vector which also includes some non-coding sequences as well. Preferably, however, non-coding sequences are excluded. Alternatively, a nucleotide sequence for a soluble form of a polypeptide may be utilized. Another preferred therapeutic viral vector includes a nucleotide sequence encoding at least

a portion of a therapeutic nucleotide sequence operatively linked to the expression vector for expression of the coding sequence in the therapeutic nucleotide sequence.

As used herein with regard to DNA sequences or segments, the phrase "operatively linked" generally means the sequences or segments have been covalently joined into one piece of DNA, whether in single or double stranded form.

The choice of viral vector into which a therapeutic nucleotide sequence of this invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, *e.g.*, vector replication and protein expression, and the host cell to be transformed -- these being limitations inherent in the art of constructing recombinant DNA molecules. Although certain adenovirus serotypes are recited herein in the form of specific examples, it should be understood that the present invention contemplates the use of *any* adenovirus serotype, including hybrids and derivatives thereof. As one will observe, it is not unusual or outside the scope of the present invention to utilize nucleotide and/or amino acid residue sequences of two or more serotypes in constructs, compositions and methods of the invention.

A translatable nucleotide sequence is a linear series of nucleotides that provide an uninterrupted series of at least 8 codons that encode a polypeptide in one reading frame. Preferably, the nucleotide sequence is a DNA sequence. The vector itself may be of any suitable type, such as a viral vector (RNA or DNA), naked straight-chain or circular DNA, or a vesicle or envelope containing the nucleic acid material and any polypeptides that are to be inserted into the cell.

2. Promoters

As noted elsewhere herein, an expression nucleic acid in an Ad-derived vector of the present invention may also include a promoter sequence.

In general, promoters are DNA segments that contain a DNA sequence that controls the expression of a gene located 3' or downstream of the promoter. The promoter is the DNA sequence to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene, typically located 3' of the promoter. A promoter also includes DNA sequences which direct the initiation of transcription, including those to which RNA polymerase specifically binds. If more than one nucleic acid sequence encoding a particular polypeptide or protein is included in a therapeutic viral vector or nucleotide sequence, more than one promoter or enhancer element may be included, particularly if that would enhance

efficiency of expression. For purposes of the present invention, regulatable (inducible) as well as constitutive promoters may be used, either on separate vectors or on the same vector.

Both constitutive and regulatable (often called "inducible") promoters are useful in constructs and methods of the present invention. For example, some useful regulatable promoters are those of the CREB-regulated gene family and include inhibin, gonadotropin, cytochrome c, glucagon, and the like. (See, *e.g.*, published International App. No. WO96/14061.

A regulatable or inducible promoter may be described as a promoter wherein the rate of RNA polymerase binding and initiation is modulated by external stimuli. (*See* U.S. Patent Nos. 5,750,396 and 5,998,205). Such stimuli include various compounds or compositions, light, heat, stress, chemical energy sources, and the like. Inducible, suppressible and repressible promoters are considered regulatable promoters.

Regulatable promoters may also include tissue-specific promoters. Tissue-specific promoters direct the expression of the gene to which they are operably linked to a specific cell type. Tissue-specific promoters cause the gene located 3' of it to be expressed predominantly, if not exclusively, in the specific cells where the promoter expressed its endogenous gene. Typically, it appears that if a tissue-specific promoter expresses the gene located 3' of it at all, then it is expressed appropriately in the correct cell types (see, *e.g.*, Palmiter *et al., Ann. Rev. Genet. 20*: 465-499, 1986).

When a tissue-specific promoter controls the expression of a gene, that gene will be expressed in a small number of tissues or cell types rather than in substantially all tissues and cell types. Examples of tissue-specific promoters include the immunoglobulin promoter described by Brinster *et al., Nature 306:* 332-336 (1983) and Storb *et al., Nature 310*: 238-231 (1984); the elastase-I promoter described by Swift *et al., Cell 38*: 639-646 (1984); the globin promoter described by Townes *et al., Mol. Cell. Biol. 5*: 1977-1983 (1985), and Magram *et al., Mol. Cell. Biol. 9*: 4581-4584 (1989), the insulin promoter described by Bucchini *et al., PNAS USA, 83*: 2511-2515 (1986) and Edwards *et al., Cell 58*: 161 (1989); the immunoglobulin promoter described by Ruscon *et al., Nature 314*: 330-334 (1985) and Grosscheld *et al., Cell 38:* 647-658 (1984); the alpha actin promoter described by Shani, *Mol. Cell. Biol. 6*: 2624-2631 (1986); the alpha crystalline promoter described by Overbeek *et al., PNAS USA 82*: 7815-7819 (1985); the prolactin promoter described by Crenshaw *et al., Genes and Development 3*: 959-972 (1989); the propiomelanocortin promoter described by Tremblay *et al.,*

PNAS USA 85: 8890-8894 (1988); the beta-thyroid stimulating hormone (BTSH) promoter described by Tatsumi *et al., Nippon Rinsho 47*: 2213-2220 (1989); the mouse mammary tumor virus (MMTV) promoter described by Muller *et al., Cell 54: 105* (1988); the albumin promoter described by Palmiter *et al., Ann. Rev. Genet. 20:* 465-499 (1986); the keratin promoter described by Vassar *et al., PNAS USA 86*: 8565-8569 (1989); the osteonectin promoter described by McVey *et al., J. Biol. Chem. 263:* 11,111-11,116 (1988); the prostate-specific promoter described by Allison *et al., Mol. Cell. Biol. 9*: 2254-2257 (1989); the opsin promoter described by Nathans *et al., PNAS USA 81*: 4851-4855 (1984); the olfactory marker protein promoter described by Danciger *et al., PNAS USA 86*: 8565-8569 (1989); the neuron-specific enolase (NSE) promoter described by Forss-Pelter *et al., J. Neurosci. Res. 16*: 141-151 (1986); the L-7 promoter described by Sutcliffe, *Trends in Genetics 3*: 73-76 (1987) and the protamine 1 promoter described Peschon *et al., Ann. New York Acad. Sci. 564*: 186-197 (1989) and Braun *et al., Genes and Development 3*: 793-802 (1989).

3. Adenovirus Vectors

Although adenovirus consists of many proteins, not all adenovirus proteins are required for assembly of a recombinant adenovirus particle (vector) of this invention. Thus, deletion of the appropriate genes from a recombinant Ad vector as taught herein will thus allow the vector to accommodate even larger "foreign" DNA segments. Thus, if the sequences encoding one or more adenovirus polypeptides or proteins are supplanted by a recombinant nucleotide sequence of the present invention, the length of the recombinant sequence can conceivably extend nearly to the packaging limit of the relevant adenovirus-derived vector.

In view of the fact that preferred embodiments disclosed herein are helper-independent Ad-derived vectors, the entire wild-type Ad genome cannot be completely supplanted by recombinant nucleic acid molecules without transforming such a vector into a vector requiring "help" of some kind. However, most of the Ad-derived vectors of the present invention do not depend on a helper virus; instead, the vectors of the present invention are propagated in cell lines stably expressing proteins or polypeptides that have been removed from said vectors to allow the addition of "foreign" DNA into the vectors. In various disclosed embodiments, specific early region and structural polypeptides are deleted from the vectors of the present invention, thereby enabling the vectors to accommodate recombinant nucleic acid sequences (or cassettes) of various lengths. For example, Ad-derived

vectors of the present invention may easily include 12 kb or more of foreign (or "therapeutic") DNA sequences.

Thus, adenovirus viral vectors are also disclosed which comprise nucleotide sequences encoding a packaging signal and a foreign protein or polypeptide, wherein the nucleotide sequence encoding an adenovirus structural protein has been deleted.

In one variation, the nucleotide sequence encoding the foreign protein or polypeptide is a DNA molecule up to about 3 kb in length; in another, the nucleotide sequence encoding the foreign protein or polypeptide is a DNA molecule up to about 9.5 kb in length; in still another, the nucleotide sequence encoding the foreign protein or polypeptide is a DNA molecule up to about 12.5 kb in length. Nucleotide sequences of intermediate lengths are also contemplated by the present invention, as are sequences in excess of 12.5 kb.

The invention also discloses viral vectors wherein the sequence encoding a foreign protein or polypeptide is a sequence encoding an anti-tumor agent, a tumor suppressor protein, a suicide protein, or a fragment or functional equivalent thereof. In one variation, nucleotide sequences encoding one or more regulatory proteins have also been deleted from the vector. In another, the regulatory proteins are selected from the group consisting of E1A, E1B, E2A, E2B, E3, E4, and L4 (100K protein).

A wide variety of therapeutic viral vectors are also embodiments of the present invention. In one embodiment, a therapeutic viral vector is disclosed which lacks a DNA sequence encoding fiber protein, or a portion thereof. In another variation, a therapeutic viral vector may further or alternatively comprise deletion of a DNA sequence encoding one or more regulatory proteins, polypeptides, or fragments thereof. In various embodiments, foreign DNA sequences are inserted in place of the DNA sequence encoding fiber protein in the viral vectors of the present invention. In other embodiments, the therapeutic viral vectors further comprise foreign DNA sequences inserted in place of the DNA sequences encoding one or more regulatory proteins, polypeptides, or fragments thereof, and/or one or more structural proteins, polypeptides, or fragments thereof.

The present invention further discloses a number of viral vectors. In one variation, a viral vector comprises a deletion or mutation of a DNA sequence encoding an adenovirus structural protein, polypeptide, or fragment thereof. A vector may further comprise deletion or mutation of the DNA sequences encoding regulatory polypeptides E1A and E1B; and it may still further comprise deletion or mutation of the DNA sequence encoding one or more of the following regulatory

proteins or polypeptides: E2A, E2B, E3, E4, L4, or fragments thereof. In another variation, in a viral vector of the present invention, the structural protein comprises fiber. Any combination of the foregoing is also contemplated by the present invention. The viral vectors of the present invention are suitable for the preparation of pharmaceutical compositions comprising any of the therapeutic viral vectors disclosed herein -- including combinations thereof -- are also disclosed herein. A further use of the viral vectors of the present invention is for targeting specific cells in a cell population comprising different cell types.

Yet another variation discloses that a foreign DNA sequence encoding one or more foreign proteins, polypeptides or fragments thereof has been inserted in place of any of the deletions in the therapeutic viral vector. In one embodiment, the foreign DNA encodes a tumor-suppressor protein or a biologically active fragment thereof. In another embodiment, the foreign DNA encodes a suicide protein or a biologically active fragment thereof.

The invention further contemplates that a viral vector comprises a foreign DNA sequence encoding one or more foreign proteins, polypeptides or fragments thereof wherein said DNA sequence has been inserted in place of any structural and/or regulatory proteins (or portions thereof) that have been deleted. Thus, in one embodiment, the foreign DNA encodes a therapeutic molecule such as a tumor-suppressor protein; a suicide protein; a cystic fibrosis transmembrane conductance regulator (CFTR) protein; or a biologically active fragment of any of them.

The therapeutic (or foreign) nucleotide sequence can be a gene or gene fragment that encodes a protein or polypeptide -- or a biologically active fragment thereof. (See, *e.g.*, Crystal, *et al., Nature Genetics 8:* 42-51 (1994); Zabner, *et al., Cell 75*: 207-216 (1993); Knowles, *et al., NEJM 333(13)*: 823-831 (1995); and Rosenfeld, *et al., Cell 68*: 143-155 (1992).

It is further contemplated that a therapeutic protein or polypeptide expressed by a therapeutic viral vector of the present invention may be used in conjunction with another therapeutic agent when appropriate -- *e.g.*, a thymidine kinase metabolite may be used in conjunction with the gene encoding thymidine kinase and its gene product -- in order to be even more effective.

Alternatively, a therapeutic viral vector can include a DNA or RNA oligonucleotide sequence that exhibits therapeutic activity without needing to be translated into a polypeptide product before exerting a therapeutic effect. Examples of the latter include antisense oligonucleotides that will inhibit the transcription of deleterious genes or ribozymes that act as site-specific

ribonucleases for cleaving selected mutated gene sequences. In another variation, a therapeutic nucleotide sequence of the present invention may comprise a DNA construct capable of generating therapeutic nucleotide molecules, including ribozymes and antisense DNA, in high copy numbers in target cells, as described in published PCT application No. WO 92/06693 (the disclosure of which is incorporated herein by reference). Other preferred therapeutic nucleotide sequences according to the present invention are capable of delivering HIV antisense oligonucleotides to latently-infected T cells via CD4. Similarly, delivery of Epstein-Barr Virus (EBV) EBNa-1 antisense oligonucleotides to B cells via CR2 is capable of effecting therapeutic results.

A preferred recombinant adenovirus vector genome is based on the vector described in the Examples and designated Ad5.βgal.ΔF. This vector is a helper independent, fiberless vector genome which can host, upon insertion, an exogenous gene for expression of an exogenous or therapeutic protein. The genome of Ad5.βgal.ΔF has a nucleotide sequence shown in SEQ ID NO: 27. A virus particle containing Ad5.βgal.ΔF vector genome has been prepared as described in the Examples and is deposited with the ATCC as Accession No. VR-2636

The Ad5.βgal.ΔF genome nucleic acid can be manipulated to contain any exogenous gene in place of the beta-galactosidase gene present in the construct, as described herein.

Construction of Therapeutic Viral Vectors for Gene Delivery

A. Adenovirus Particles

Various methods of making and using the vectors, plasmids, cell lines and other compositions and constructs of the present invention are also disclosed herein. The following methods are considered exemplary and not limiting.

Thus, in one variation, the invention discloses a method of constructing therapeutic viral vectors, comprising introducing a delivery plasmid into an Ad fiber-expressing complementing cell line, wherein the DNA sequence encoding Ad fiber protein has been deleted from the delivery plasmid. In one variation, the delivery plasmid further includes a DNA sequence encoding a foreign protein, polypeptide, or fragment thereof. In other embodiments, a combination of pDV44 and pΔE1Bβgal or a similar construct such as, for example, that found in pDV44, pΔE1Bβgal or the equivalent.

A recombinant adenovirus particle may be produced with a fiber protein, or it may be produced without a fiber protein ("fiberless particle") according to the present invention. Where the particle is made without fiber, such as by passaging the fiberless viral vector genome, e.g., Ad5.βgal.ΔF in the 293 cells, a fiberless genome is replicated and packaged in a fiberless particle. In contrast, where the fiberless genome Ad5.βgal.ΔF is passaged in the 211B or other fiber expressing cells, a fiberless genome is replicated and packaged into a fiber-containing particle.

Recombinant adenovirus particles may be made such that they include no fiber proteins, modified fiber proteins or other exogenous proteins. They may also be produced in systems using either helper-independent or helper-dependent adenovirus recombinant genomes, i.e. with or without helper viruses.

B. Targeting of Particles to Tissues - Virus Trophism

A preferred viral vector particle in which therapeutic nucleotide compositions of this invention are present is derived from adenovirus (Ad). As taught herein, viral vector particles of this invention may be designed and constructed in such a way that they specifically target a preselected recipient cell type, depending on the nature of therapy one seeks to administer. Methods of making and using therapeutic viral vectors that target specific cells are further described in the Examples that follow.

Novel vectors, viral particles or compositions may also be designed and prepared to preferentially target cells that might not otherwise be targeted by wild-type adenovirus virions. For example, in order to target non-epithelial cells, one following the teachings of the present specification may be able to prepare a therapeutic vector particle including a nucleotide sequence encoding a foreign protein, polypeptide or other ligand directed to a non-epithelial cell or to a different receptor than that generally targeted by a particular adenovirus. Examples of useful ligands directed to specific receptors (identified in parentheses) include the V3 loop of HIV gp120 (CD4); transferring (transferrin receptor); LDL, apolipoprotein B100, apolipoprotein E (LDL receptors); and deglycosylated proteins (asialoglycoprotein receptor). Various useful ligands which may be added to adenovirus fiber -- and methods for preparing and attaching same -- are set forth in U.S. Patent Nos. 5,756,086 and 5,543,328.

In yet another embodiment, the non-native amino acid residue sequence is incorporated into the fiber amino acid residue sequence at a location other than

one of the fiber termini. Alternatively, the non-native amino acid residue sequence alters the binding specificity of the fiber for a targeted cell type. In other embodiments, the linker sequence alters the binding specificity of the fiber for a targeted cell type. The expressed fiber may, in various embodiments, bind to a specific targeted cell type not usually targeted by adenovirus and/or may comprise amino acid residue sequences from more than one adenovirus serotype.

Useful ligands may be encoded by a foreign nucleotide sequence contained within a viral vector of the present invention, or may be linked to proteins or polypeptides, include antibodies and attachment sequences, as well as receptors themselves. For example, antibodies to cell receptor molecules such as integrins and the like, MHC Class I and Class II, asialoglycoprotein receptor, transferrin receptors, LDL receptors, CD4, and CR2 are but a few which are useful according to the present invention. It is also understood that the ligands typically bound by receptors, as well as analogs to those ligands, may be used as cellular targeting agents, as disclosed herein.

Therapeutic Methods

The recombinant adenovirus vectors of the present invention, typically in the form of an adenovirus particle encapsulating a recombinant adenovirus vector genome containing an expression cassette for expressing a therapeutic gene, are particularly suited for gene therapy. Thus, various therapeutic methods are contemplated by the present invention.

For example, it has now been discovered that Ad-derived viral vectors are capable of delivering a therapeutic nucleotide sequence to a specific cell or tissue, based on the tissue tropism of the particle, thereby expanding and enhancing treatment options available in numerous conditions in which more conventional therapies are of limited efficacy. Accordingly, methods of gene therapy utilizing a recombinant adenovirus particle containing a modified fiber or chimeric fiber which targets a preselected tissue, as described herein, is within the scope of the invention. Vector particles are typically purified and then an effective amount is administered *in vivo* or *ex vivo* (*in vitro*)into the subject.

For *in vitro* or *ex vivo* gene transfer, administration is often accomplished by first isolating a selected cell population from a patient such as lung epithelial cells, lymphocytes and the like followed by *in vitro* or ex vivo gene transfer of the therapeutic compositions of this invention and the replacement of the cells into the patient. *In vivo* therapy is also contemplated, *e.g.*, via the administration of

therapeutic compositions of this invention by various delivery means. For example, aerosol administration and administration via subcutaneous, intravenous, intraperitoneal, intramuscular, ocular means and the like are also within the scope of the present invention.

Other gene-delivery methods are also useful in conjunction with the methods, compositions and constructs of the present invention; see, *e.g.*, published International Application No. WO 95/11984.

The present invention also contemplates various methods of targeting specific cells -- *e.g.*, cells in a subject in need of diagnosis and/or treatment. As discussed herein, the present invention contemplates that the viral vectors and compositions of the present invention may be directed to specific receptors or cells, for the ultimate purpose of delivering those vectors and compositions to specific cells or cell types. The viral vectors and constructs of the present invention are particularly useful in this regard.

In general, adenovirus attachment and uptake into cells are separate but cooperative events that result from the interaction of distinct viral coat proteins with a receptor for attachment and α_V integrin receptors for internalization. Adenovirus attachment to the cell surface via the fiber coat proteins has been discovered to be dissociable and distinct from the subsequent step of internalization, and the present invention is able to take advantage of and function in conjunction with these differing receptors.

The invention also discloses methods of transforming a pathologic hyperproliferative mammalian cell comprising contacting the cell with any of the vectors described herein. In another embodiment, methods of infecting a mammalian target cell with a viral vector containing a preselected foreign nucleotide sequence are disclosed. One such variation comprises the following steps: (a) infecting the target cell with a viral vector of the present invention, the viral vector carrying a preselected foreign nucleotide sequence; and (b) expressing the foreign nucleotide sequence in the targeted cell.

The invention also encompasses mammalian target cells infected with a preselected foreign nucleotide sequence produced by the methods disclosed herein. In one variation, the target cells are selected from the group consisting of replicating, slow-replicating and non-replicating human cells.

Methods of treating an acquired or hereditary disease are also disclosed. One method comprises (a) administering a pharmaceutically acceptable dose of a viral vector to a target cell, wherein the vector comprises a preselected therapeutic nucleotide sequence; and (b) expressing the therapeutic sequence in the target

cell for a time period sufficient to ameliorate the acquired or hereditary disease in the cell. Method of gene therapy comprising administering to a subject an effective amount of a therapeutic viral vector produced by a packaging cell line of the present invention are also disclosed.

Also contemplated by the present invention are various methods of inhibiting the proliferation of a tumor in a subject comprising administering an effective amount of a therapeutic viral vector of the present invention under suitable conditions to the subject. In one variation, the gene encodes an anti-tumor agent. In another variation, the agent is a tumor-suppressor gene. In still another embodiment, the agent is a suicide gene or a functional equivalent thereof. In another variation, the vector is administered via intra-tumoral injection.

A composition of this invention may be used prophylactically or therapeutically *in vivo* to disrupt HIV infection and mechanisms of action by inhibiting gene expression or activation, via delivery of antisense HIV sequences or ribozymes to T cells or monocytes. Using methods of the present invention, one may target therapeutic viral vectors as disclosed herein to specific cells and tissues, including hematopoietic cells, as infection of such cells appears to be mediated by distinct integrins to which viral vectors of the present invention may readily be targeted. (See, *e.g.*, Huang, *et al., J. Virol. 70*: 4502-8, 1996).

Other useful therapeutic nucleotide sequences include antisense nucleotide sequences complementary to EBV EBNa-1 gene. Use of such therapeutic sequences may remediate or prevent latent infection of B cells with EBV. As discussed herein and in the Examples below, targeting and delivery may be accomplished via the use of various ligands, receptors, and other appropriate targeting agents.

Thus, in one embodiment, a therapeutic method of the present invention comprises contacting the cells of a subject infected with EBV or HIV with a therapeutically effective amount of a pharmaceutically acceptable composition comprising a therapeutic nucleotide sequence of this invention. In a related embodiment, the contacting involves introducing the therapeutic nucleotide sequence composition into cells having an EBV or HIV-mediated infection.

Methods of gene therapy are well known in the art (see, *e.g.*, Larrick and Burck, *Gene Therapy: Application of Molecular Biology*, Elsevier Science Publ. Co., Inc., New York, NY (1991); Kriegler, *Gene Transfer and Expression: A Laboratory Manual*, W. H. Freeman and Company, New York, 1990). The term "subject" should be understood to include any animal -- particularly mammalian --

patient, such as any murine, rat, bovine, porcine, canine, feline, equine, ursine, or human patient.

When the foreign gene carried in the vector encodes a tumor suppressor gene or another anti-tumor protein, the vector is useful to treat or reduce hyperproliferative cells in a subject, to inhibit tumor proliferation in a subject or to ameliorate a particular, related pathology.

The present invention also contemplates methods of depleting suitable samples of pathologic mammalian hyperproliferative cells contaminating hematopoietic precursors during bone marrow reconstitution via the introduction of a wild-type tumor suppressor gene into the cell preparation using a vector of this invention. As used herein, a suitable sample is defined as a heterogeneous cell preparation obtained from a patient, *e.g.*, a mixed population of cells containing both phenotypically normal and pathogenic cells.

Administration includes -- but is not limited to -- the introduction of therapeutic agents of the present invention into a cell or subject via various means, including direct injection, intravenously, intraperitoneally, via intra-tumor injection, via aerosols, or topically. Therapeutic agents as disclosed herein may also be combined for administration of an effective amount of the agents with a pharmaceutically-acceptable carrier, as described herein.

As used herein, "effective amount" generally means the amount of vector particle (or proteins produced/released thereby) which achieves a positive outcome in the subject to whom the vector is administered. The total volume administered will necessarily vary depending on the mode of administration, as those of skill in the relevant art will appreciate, and dosages may vary as well.

The dose of a biologic vector (particle) is somewhat complex and may be described in terms of the concentration (in plaque-forming units per milliliter (pfu/ml)), the total dose (in pfus), or the estimated number of particles administered per cell (the estimated multiplicity of infection or MOI). Thus, if a vector is administered via infusion -- say, across nasal epithelium -- at a constant total volume, the respective concentration, etc. may be described as follows:

In general, when recombinant adenoviral vector particles are administered via infusion across the nasal epithelium (e.g. an area of nasal epithelium containing 2 x 10^7 cells,) administered amounts producing an estimated MOI (multiplicity of infection) of about 10 or greater are much more effective than lower

Concentration (pfu/ml)	Volume (ml)	Dose (pfu)	Estimated MOI
107	2	2×10^7	1
108	2	2×10^8	10
109	2	2×10^9	100
1010	2	2×10^{10}	1000

Table 2

dosages. (See, *e.g.*, Knowles, *et al., New Eng. J. Med. 333:* 823-831, 1995). Similarly, when direct injection is the preferred treatment modality -- *e.g.*, direct injection of a viral vector into a tumor -- doses of 1 x 10^9 pfu or greater are generally preferred. (See, *e.g.*, published International App. No. WO95/11984.)

Thus, depending on the mode of administration, an effective amount administered in a single dose preferably contains from about 10^6 to about 10^{15} infectious units. A typical course of treatment would be one such dose per day over a period of five days. As those of skill in the art will appreciate, an effective amount may vary depending on (1) the pathology or other condition to be treated, (2) the status and sensitivity of the patient, and (3) various other factors well known to those of skill in the art, such as the patient's tolerance to other courses of treatment that may have been applied previously. Thus, those of skill in the art may easily and precisely determine effective amounts of the agents/vectors of the present invention which may be administered to a particular patient, based on their understanding of and evaluation of such factors.

The present invention also contemplates methods of ameliorating pathologies characterized by hyperproliferative cells or genetic defects in a subject, by administering to the subject an effective amount of a vector as described herein. Such vectors preferably contain a foreign gene encoding a gene product (*e.g.* polypeptide or protein) having the ability to ameliorate the pathology, under suitable conditions. As used herein, the term "genetic defect" means any disease,

condition or abnormality which results from inherited factors, *e.g.* Huntington's Disease, Tay-Sachs Disease, or Sickle Cell Disease.

The present invention further provides methods for reducing the proliferation of tumor cells in a subject by introducing into the tumor mass an effective amount of an adenoviral expression vector containing an anti-tumor gene other than a tumor suppressor gene. The anti-tumor gene can encode, for example, thymidine kinase (TK). An effective amount of a therapeutic agent is then administered to the subject; the therapeutic agent, in the presence of the anti-tumor gene, is toxic to the cell.

Using thymidine kinase as exemplary, the therapeutic agent is a thymidine kinase metabolite such as ganciclovir (GCV), 6-methoxypurine arabinonucleoside (araM), or a functional equivalent thereof. Both the thymidine kinase gene and the thymidine kinase substrate must be used concurrently in order to exert a toxic effect on the host cell. In the presence of the TK gene, GCV is phosphorylated and becomes a potent inhibitor of DNA synthesis, whereas araM is converted to the cytotoxic anabolite araATP. Thus, the precise method of action or synergism is not relevant to therapeutic efficacy; what is relevant is the fact that the concurrent use of appropriate genes and therapeutic agents may effectively ameliorate a specific disease condition.

Another useful example contemplates use of a vector of the present invention which expresses the enzyme cytosine deaminase. Such a vector could be used in conjunction with administration of the drug 5-fluorouracil (Austin and Huber, *Mol. Pharm. 43*: 380-387, 1993) or the recently-described *E. coli* Deo gene in combination with 6-methyl-purine-2'-deoxyribonucleoside (Sorscher *et al., Gene Therapy 1:* 233-238, 1994).

As with the use of the tumor suppressor genes described previously, the use of other anti-tumor genes, either alone or in combination with the appropriate therapeutic agent, provides a treatment for the uncontrolled cell growth or proliferation characteristic of tumors and malignancies. Thus, the present invention provides therapies to halt the uncontrolled cellular growth in a patient, thereby alleviating the symptoms or the disease or cachexia present in the patient. The effect of this treatment includes, but is not limited to, prolonged survival time of the patient, reduction in tumor mass or burden, apoptosis of tumor cells, or the reduction in the number of circulating tumor cells. Means of quantifying the beneficial effects of this therapy are well known to those of skill in the art.

The present invention provides a recombinant adenovirus expression vector characterized by the partial or total deletion of one or more adenoviral structural

protein genes, such as the gene encoding fiber, which allows the vector to accommodate a therapeutic, foreign nucleic acid sequence encoding a functional foreign polypeptide, protein, or biologically active fragment thereof. A therapeutic gene sequence may be introduced into a tumor mass by combining the adenoviral expression vector with a suitable pharmaceutically acceptable carrier. Introduction can be accomplished, for example, via direct injection of the recombinant Ad vector into the tumor mass.

A method of tumor-specific delivery of a tumor-suppressor gene is accomplished by contacting target tissue in a subject with an effective amount of a recombinant Ad-derived vector of this invention. In the case of anti-tumor therapy, the gene is intended to encode an anti-tumor agent, such as a functional tumor suppressor gene product or suicide gene product. The term "contacting" is intended to encompass any delivery method for the efficient transfer of the vector, such as via intra-tumoral injection.

In another example, adenovirus vectors of the present invention can be used to transfer genes to central nervous system (CNS) tumors *in vivo*.

The present invention also contemplates methods for determining the efficacy of the within-disclosed therapeutic compositions and methods. One such method for confirming efficacy utilizes the human/SCID (severe combined immunodeficient) mouse model of EBV-induced LPD (lymphoproliferative disease) to ascertain whether EBV-antisense therapeutic nucleotide sequences block tumor formation. (See, *e.g.*, Pisa, *et al., Blood 79*: 173-179 (1992); Rowe, *et al., Curr. Top. Microbiol. Immunol. 166*: 325 (1990); and Cannon, *et al.*, J. *Clin. Invest. 85*: 1333-1337 (1990).

Finally, the use of Ad vectors of the present invention to prepare medicaments for the treatment, therapy and/or diagnosis of various diseases is also contemplated by this invention. Moreover, other anti-tumor genes may be used in combination with the corresponding therapeutic agent to reduce the proliferation of tumor cells. Such other gene-and-therapeutic-agent combinations are known to those of skill in the art and may be applied as taught herein.

A. Therapeutic Compositions

In various alternative embodiments of the present invention, therapeutic sequences and compositions useful for practicing the therapeutic methods described herein are contemplated. Therapeutic compositions of the present invention may contain a physiologically tolerable carrier together with one or more

therapeutic nucleotide sequences of this invention, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the composition is not immunogenic or otherwise able to cause undesirable side effects when administered to a subject for therapeutic purposes.

As used herein, the terms "pharmaceutically acceptable," "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a subject -- *e.g.*, a mammal -- without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

For example, the present invention comprises therapeutic compositions useful in the specific targeting of epithelial or non-epithelial cells as well as in delivering a therapeutic nucleotide sequence to those cells. Therapeutic compositions designed to preferentially target to epithelial cells may comprise a recombinant adenovirus-derived vector particle including a therapeutic nucleotide sequence. As described herein, a number of adenovirus-derived moieties are described, including particles lacking fiber, particles that contain wild type adenovirus fiber, and particles that contain modified or chimeric fiber, each type providing a different tissue tropism to the particle.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art. Typically such compositions are prepared as injectables -- either as liquid solutions or suspensions -- however, solid forms suitable for solution or suspension in liquid prior to use can also be prepared. A preparation can also be emulsified, or formulated into suppositories, ointments, creams, dermal patches, or the like, depending on the desired route of administration.

Physiologically tolerable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

A therapeutic composition typically contains an amount of a therapeutic material, *i.e.*, a nucleotide sequence or adenovirus vector particle of the present invention, sufficient to deliver a therapeutically effective amount to the target tissue, typically an amount of at least 0.1 weight percent to about 90 weight percent of therapeutic material per weight of total therapeutic composition. A weight percent is a ratio by weight of therapeutic material, *e.g.*, a nucleotide sequence, to total composition. Thus, for example, 0.1 weight percent is 0.1 grams of DNA segment per 100 grams of total composition.

Other Applications

The cell lines, viral vectors and methods of the present invention may also be used for purposes other than the direct administration of therapeutic nucleotide sequences. In one such application, the production of large quantities of biologically active proteins or polypeptides in cells transfected with the within-disclosed viral vectors is contemplated herein. For example, human lymphoblastoid cells may be transfected with a viral vector of the present invention carrying a human hematopoietic growth factor such as the gene for erythropoietin (EPO); cells so transfected are thus able to produce biologically active EPO. (See, *e.g.*, Lopez *et al., Gene 148*: 285-91, 1994).

Various other applications and uses of the within-described methods, cell lines, plasmids, vectors, and compositions of the present invention shall become apparent upon closer examination of the Examples that follow.

The following examples are intended to illustrate, but not limit, the present invention. As such, the following description provides details of the manner in which particular embodiments of the present invention may be made and used. This description, while exemplary of the present invention, is not to be construed as specifically limiting the invention. Variations and equivalents, now known or later developed, which would be within the understanding and technical competence of one skilled in this art are to be considered as falling within the scope of this invention.

Example 1

Preparation of Adenovirus Packaging Cell Lines

Cell lines that are commonly used for growing adenovirus are useful as host cells for the preparation of adenovirus packaging cell lines. Preferred cells include 293 cells, an adenovirus-transformed human embryonic kidney cell line obtained from the ATCC, having Accession Number CRL 1573; HeLa, a human epithelial carcinoma cell line (ATCC Accession Number CCL-2); A549, a human lung carcinoma cell line (ATCC Accession Number CCL 1889); and the like epithelial-derived cell lines. As a result of the adenovirus transformation, the 293 cells contain the E1 early region regulatory gene. All cells were maintained in complete DMEM + 10% fetal calf serum unless otherwise noted.

The cell lines of this invention allow for the production and propagation of novel adenovirus-based gene delivery vectors having deletions in preselected gene regions, that are obtained by cellular complementation of adenoviral genes. To provide the desired complementation of such deleted adenoviral genomes in order to generate a novel viral vector of the present invention, plasmid vectors that contain preselected functional units were designed as described herein. Such units include but are not limited to E1 early region, E4 and the viral fiber gene. The preparation of plasmids providing such complementation, thereby being "complementary plasmids or constructs," that are stably inserted into host cell chromosomes are described below.

A. Preparation of an E4-Expressing Plasmid for Complementation of E4-Gene-Deleted Adenoviruses

The viral E4 regulatory region contains a single transcription unit which is alternately spliced to produce several different mRNAs. The E4-expressing plasmid prepared as described herein and used to transfect the 293 cell line contains the entire E4 transcriptional unit as shown in Figure 1. A DNA fragment extending from 175 nucleotides upstream of the E4 transcriptional start site including the natural E4 promoter to 153 nucleotides downstream of the E4 polyadenylation signal including the natural E4 terminator signal, corresponding to nucleotides 32667-35780 of the adenovirus type 5 (hereinafter referred to as Ad5) genome as described in Chroboczek *et al.* (*Virol.*, 186:280-285 (1992), GenBank Accession Number M73260), was amplified from Ad5 genomic DNA, obtained from the ATCC, via the polymerase chain reaction (PCR). Sequences of the primers used were 5'CGGTACACAGAATTCAGGAGACACAACTCC3' (forward or 5' primer referred to as E4L) (SEQ ID NO: 1) and 5'GCCTGGATCCGGGAAGTTACGTAACGTGGGAAAAC3' (SEQ ID NO: 2)

(backward or 3' primer referred to as E4R). To facilitate cloning of the PCR fragment, these oligonucleotides were designed to create novel sites for the restriction enzymes EcoRI and BamHI, respectively, as indicated with underlined nucleotides. DNA was amplified via PCR using 30 cycles of 92 C for 1 minute, 50 C for 1 minute, and 72 C for 3 minutes resulting in amplified full-length E4 gene products.

The amplified DNA E4 products were then digested with EcoRI and BamHI for cloning into the compatible sites of pBluescript/SK+ by standard techniques to create the plasmid pBS/E4. A 2603 base pair (bp) cassette including the herpes simplex virus thymidine kinase promoter, the hygromycin resistance gene, and the thymidine kinase polyadenylation signal was excised from the plasmid pMEP4 (Invitrogen, San Diego, CA) by digestion with FspI followed by addition of BamHI linkers (5'CGCGGATCCGCG3') (SEQ ID NO: 3) for subsequent digestion with BamHI to isolate the hygromycin-containing fragment. The isolated BamHI-modified fragment was then cloned into the BamHI site of pBS/E4 containing the E4 region to create the plasmid pE4/Hygro containing 8710 bp (Figure 2). The pE4/Hygro plasmid has been deposited with the ATCC as described in Example 3. The complete nucleotide sequence of pE4/Hygro is listed in SEQ ID NO: 4. Position number 1 of the linearized vector corresponds to approximately the middle portion of the pBS/SK+ backbone as shown in Figure 2 as a thin line between the 3' BamHI site in the hygromycin insert and the 3' EcoRI site in the E4 insert. The 5' and 3' ends of the E4 gene are located at respective nucleotide positions 3820 and 707 of SEQ ID NO: 4 while the 5' and 3' ends of the hygromycin insert are located at respective nucleotide positions 3830 and 6470. In the clone that was selected for use, the E4 and hygromycin resistance genes were divergently transcribed.

B. Preparation of a Fiber-Expressing Plasmid for Complementation of Fiber-Gene-Deleted Adenoviruses

To prepare a fiber-encoding construct, primers were designed to amplify the fiber coding region from Ad5 genomic DNA with the addition of unique BamHI and NotI sites at the 5' and 3' ends of the fragment, respectively. The Ad5 nucleotide sequence is available with the GenBank Accession Number M18369. The 5' and 3' primers had the respective nucleotide sequences of 5'ATGGGATCCAAGATGAAGCGCGCAAGACCG3' (SEQ ID NO: 5) and 5'CATAACGCGGCCGCTTCTTTATTCTTGGGC3' (SEQ ID NO: 6), where the inserted BamHI and NotI sites are indicated by underlining. The 5' primer also contained a nucleotide substitution 3 nucleotides 5' of the second ATG codon (C to A) that is the initiation site. The nucleotide substitution was included so as to improve the consensus for initiation of fiber protein translation.

The amplified DNA fragment was inserted into the BamHI and NotI sites of pcDNA3 (Invitrogen) to create the plasmid designated pCDNA3/Fiber having 7148 bp, the plasmid map of which is shown in Figure 3. The parent plasmid contained the CMV promoter, the bovine growth hormone (BHG) terminator and the gene for conferring neomycin resistance. The viral sequence included in this construct corresponds to nucleotides 31040-32791 of the Ad5 genome.

The complete nucleotide sequence of pCDNA3/Fiber is listed in SEQ ID NO: 7 where the nucleotide position 1 corresponds to approximately the middle of the pcDNA3 vector sequence. The 5' and 3' ends of the fiber gene are located at respective nucleotide positions 916 with ATG and 2661 with TAA.

To enhance expression of fiber protein by the constitutive CMV promoter provided by the pcDNA vector, a BglII fragment containing the tripartite leader (TPL) of adenovirus type 5 was excised from pRD112a (Sheay *et al.*, *BioTechniques*, 15:856-862 (1993) and inserted into the BamHI site of pCDNA3/Fiber to create the plasmid pCLF having 7469 bp, the plasmid map of which is shown in Figure 4. The adenovirus tripartite leader sequence, present at the 5' end of all major late adenoviral mRNAs as described by Logan *et al.*, *Proc. Natl. Acad. Sci., USA*, 81:3655-3659 (1984) and Berkner, *BioTechniques, 6*:616-629 (1988), also referred to as a "partial TPL" since it contains a partial exon 1, shows correspondence with the Ad5 leader sequence having three spatially separated exons corresponding to nucleotide positions 6081-6089 (the 3' end of the first leader segment), 7111-7182 (the entire second leader segment), and

9644-9845 (the third leader segment and sequence downstream of that segment). The corresponding cDNA sequence of the partial tripartite leader sequence present in pCLF is listed in SEQ ID NO: 8 bordered by BamHI/BglII 5' and 3' sites at respective nucleotide positions 907-912 to 1228-1233. The nucleotide sequence of an isolated partial TPL of the present invention is also listed separately as SEQ ID NO: 26 with the noted 5' and 3' restriction sites and with the following nucleotide regions identified: 1-6 nt BglII site; 1-18 nt polylinker; 19-27 nt last 9 nt of the first leader segment (exon 1); 28-99 nt second leader segment (exon 2); 100-187 nt third leader segment (exon 3); 188-301 nt contains the nt sequence immediately following the third leader in the genome with an unknown function; and 322-327 nt BglII site.

The pCLF plasmid has been deposited with the ATCC as described in Example 3. The complete nucleotide sequence of pCLF is listed in SEQ ID NO: 8 where the nucleotide position 1 corresponds to approximately the middle of the pcDNA3 parent vector sequence. The 5' and 3 ends of the Ad5 fiber gene are located at respective nucleotide positions 1237-1239 with ATG and 2980-2982 with TAA. The rest of the vector construct has been previously described above.

C. Generation of an Adenovirus Packaging Cell Line Carrying Plasmids Encoding Functional E4 and Fiber Proteins

The 293 cell line was selected for preparing the first adenovirus packaging line as it already contains the E1 gene as prepared by Graham *et al.*, *J. Gen. Virol.*, *36*:59-74 (1977) and as further characterized by Spector, *Virol.*, *130*:533-538 (1983). Before electroporation, 293 cells were grown in RPMI medium + 10% fetal calf serum. Four x 10^6 cells were electroporated with 20 µg each of pE4/Hygro DNA and pCLF DNA using a BioRad GenePulser and settings of 300 V, 25 µF. DNA for electroporation was prepared using the Qiagen system according to the manufacturer's instructions (Bio-Rad, Richmond, CA).

Following electroporation, cells were split into fresh complete DMEM + 10% fetal calf serum containing 200 µg/ml Hygromycin B (Sigma, St. Louis, MO).

From expanded colonies, genomic DNA was isolated using the "MICROTURBOGEN" system (Invitrogen) according to manufacturer's instructions. The presence of integrated E4 DNA was assessed by PCR using the primer pair E4R and ORF6L (5'TGCTTAAGCGGCCGCGAAGGAGA AGTCC3') (SEQ ID NO: 9), the latter of which is a 5' forward primer near adenovirus 5 open reading frame 6. Refer to Figure 1 for position of the primers relative to the E4 genes.

One clone, designated 211, was selected exhibiting altered growth properties relative to that seen in parent cell line 293. The 211 clone contained the expected product, indicating the presence of inserted DNA corresponding to most, if not all, of the E4 fragment contained in the pE4/Hygro plasmid. The 211 cell line has been deposited with the ATCC as described in Example 3. This line was further evaluated by amplification using the primer pair E4L/E4R described above, and a product corresponding to the full-length E4 insert was detected. Genomic Southern blotting was performed on DNA restricted with EcoRI and BamHI. The E4 fragment was then detected at approximately one copy/genome compared to standards with the EcoRI/BamHI E4 fragment as cloned into pBS/E4 for use as a labeled probe with the Genius system according to manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN). In DNA from the 211 cell line, the expected labeled internal fragment pE4/Hygro hybridized with the isolated E4 sequences. In addition, the probe hybridized to a larger fragment which may be the result of a second insertion event (Figure 5).

Although the 211 cell line was not selected by neomycin resistance, thus indicating the absence of fiber gene, to confirm the lack of fiber gene, the 211 cell line was analyzed for expression of fiber protein by indirect immunofluorescence with an anti-fiber polyclonal antibody and a FITC-labeled anti-rabbit IgG (KPL) as secondary. No immunoreactivity was detected. Therefore, to generate 211 clones containing recombinant fiber genes, the 211 clone was expanded by growing in RPMI medium and subjected to additional electroporation with the fiber-encoding pCLF plasmid as described above.

Following electroporation, cells were plated in DMEM + 10% fetal calf serum and colonies were selected with 200 µg/ml G418 (Gibco, Gaithersburg, MD). Positive cell lines remained hygromycin resistant. These candidate sublines of 211 were then screened for fiber protein expression by indirect immunofluorescence as described above. The three sublines screened, 211A, 211B and 211R, along with a number of other sublines, all exhibited nuclear staining qualitatively comparable to the positive control of 293 cells infected with AdRSVβgal (1 pfu/cell) and stained 24 hours post-infection.

Lines positive for nuclear staining in this assay were then subjected to Western blot analysis under denaturing conditions using the same antibody. Several lines in which the antibody detected a protein of the expected molecular weight (62 kd for the Ad5 fiber protein) were selected for further study including 211A, 211B and 211R. The 211A cell line has been deposited with ATCC as described in Example 3.

Immunoprecipitation analysis using soluble nuclear extracts from these three cell lines and a seminative electrophoresis system demonstrated that the fiber protein expressed is in the functional trimeric form characteristic of the native fiber protein as shown in Figure 6. The predicted molecular weight of a trimerized fiber is 186 kd. The lane marked 293 lacks fiber while the sublines contain detectable fiber. Under denaturing conditions, the trimeric form was destroyed resulting in detectable fiber monomers as shown in Figure 6. Those clones containing endogenous E1, newly expressed recombinant E4 and fiber proteins were selected for use in complementing adenovirus gene delivery vectors having the corresponding adenoviral genes deleted as described in Example 2.

D. Preparation of an E1-Expressing Plasmid for Complementation of E1-Gene-Deleted Adenoviruses

In order to prepare adenoviral packaging cell lines other than those based on the E1-gene containing 293 cell line as described in Example 1C above, plasmid vectors containing E1 alone or in various combinations with E4 and fiber genes are constructed as described below.

The region of the adenovirus genome containing the E1a and E1b gene is amplified from viral genomic DNA by PCR as previously described. The primers used are E1L, the 5' or forward primer, and E1R, the 3' or backward primer, having the respective nucleotide sequences 5'CCG AGCTAGC GACTGAAAATGAG3' (SEQ ID NO: 10) and 5'CCTCTCGAG AGACAGC AAGACAC3' (SEQ ID NO: 11). The E1L and E1R primers include the respective restriction sites NheI and XhoI as indicated by the underlines. The sites are used to clone the amplified E1 gene fragment into the NheI/XhoI sites in pMAM commercially available from Clontech (Palo Alto, CA) to form the plasmid pDEX/E1 having 11152 bp, the plasmid map of which is shown in Figure 7.

The complete nucleotide sequence of pDEX/E1 is listed in SEQ ID NO: 12 where the nucleotide position 1 corresponds to approximately 1454 nucleotides from the 3' end of the pMAM backbone vector sequence. The pDEX/E1 plasmid includes nucleotides 552 to 4090 of the adenovirus genome positioned downstream (beginning at nucleotide position 1460 and ending at 4998 in the pDEX/E1 plasmid) of the glucocorticoid-inducible mouse mammary tumor virus (MMTV) promoter of pMAM. The pMAM vector contains the E. coli *gpt* gene that allows stable transfectants to be isolated using

hypoxanthine/aminopterin/thymidine (HAT) selection. The pMAM backbone occupies nucleotide positions 1-1454 and 5005-11152 of SEQ ID NO: 12.

E. Generation of an Adenovirus Packaging Cell Line Carrying Plasmids Encoding Functional E1, and Fiber Proteins

To create separate adenovirus packaging cell lines equivalent to that of the 211 sublines, 211A, 211B and 211R, as described in Example 1C, alternative cell lines lacking adenoviral genomes are selected for transfection with the plasmid constructs as described below. Acceptable host cells include A549, Hela, Vero and the like cell lines as described in Example 1. The selected cell line is transfected with the separate plasmids, pDEX/EI and pCLF, respectively for expressing E1, and fiber complementary proteins. Following transfection procedures as previously described, clones containing stable insertions of the two plasmids are isolated by selection with neomycin and HAT. Integration of full-length copy of the E1 gene is assessed by PCR amplification from genomic DNA using the primer set E1L/E1R , as described above. Functional insertion of the fiber gene is assayed by staining with the anti-fiber antibody as previously described.

The resultant stably integrated cell line is then used as a packaging cell system to complement adenoviral gene delivery vectors having the corresponding adenoviral gene deletions as described in Example 2.

F. Preparation of a Plasmid Containing Two or More Adenoviral Genes for Complementing Gene-Deleted Adenoviruses

The methods described in the preceding Examples rely on the use of two plasmids, pE4/Hygro and pCLF, or, pCLF and pDEX/E1 for generating adenoviral cell packaging systems. In alternative embodiments contemplated for use with the methods of this invention, complementing plasmids containing two or more adenoviral genes for expressing of encoded proteins in various combinations are also prepared as described below. The resultant plasmids are then used in various cell systems with delivery plasmids having the corresponding adenoviral gene deletions. The selection of packaging cell, content of the delivery plasmids and

content of the complementing plasmids for use in generating recombinant adenovirus viral vectors of this invention thus depends on whether other adenoviral genes are deleted along with the adenoviral fiber gene, and, if so, which ones.

1. Preparation of a Complementing Plasmid Containing Fiber and E1 Adenoviral Genes

A DNA fragment containing sequences for the CMV promoter, adenovirus tripartite leader, fiber gene and bovine growth hormone terminator is amplified from pCLF prepared in Example 1B using the forward primer 5'GACGGATCGGGAGATCTCC3' (SEQ ID NO: 13), that anneals to the nucleotides 1-19 of the pCDNA3 vector backbone in pCLF, and the backward primer 5'CCGCCTCAGAAGCCATAGAGCC3' (SEQ ID NO: 14) that anneals to nucleotides 1278-1257 of the pCDNA3 vector backbone. The fragment is amplified as previously described and then cloned into the pDEX/E1 plasmid, prepared in Example 1D. For cloning in the DNA fragment, the pDEX/E1 vector is first digested with NdeI, that cuts at a unique site in the pMAM vector backbone in pDEX/E1, then the ends are repaired by treatment with bacteriophage T4 polymerase and dNTPs.

The resulting plasmid containing E1 and fiber genes, designated pE1/Fiber, provides both dexamethasone-inducible E1 function as described for DEX/E1 and expression of Ad5 fiber protein as described above. A schematic plasmid map of pE1/Fiber, having 14455 bp, is shown in Figure 8.

The complete nucleotide sequence of pE1/Fiber is listed in SEQ ID NO: 15 where the nucleotide position 1 corresponds to approximately to 1459 nucleotides from the 3' end of the parent vector pMAM sequence. The 5' and 3 ends of the Ad5 E1 gene are located at respective nucleotide positions 1460 and 4998 followed by pMAM backbone and then separated from the Ad5 fiber from pCLF by the filled-in blunt ended NdeI site. The 5' and 3' ends of the pCLF fiber gene fragment are located at respective nucleotide positions 10922-14223 containing elements as previously described for pCLF.

The resultant pE1/Fiber plasmid is then used to complement one or more delivery plasmids expressing E1 and fiber.

The pE1/Fiber construct is then used to transfect a selected host cell as described in Example 1E to generate stable chromosomal insertions preformed as previously described followed by selection on HAT medium. The stable cells are then used as packaging cells as described in Example 2.

2. Preparation of a Complementing Plasmid Containing E4 and Fiber Adenoviral Genes

pCLF prepared as described in Example 1B is partially digested with BglII to cut only at the site in the pCDNA3 backbone. The pE4/Hygro plasmid prepared in Example 1A is digested with BamHI to produce a fragment containing E4. The E4 fragment is then inserted into the BamHI site of pCLF to form plasmid pE4/Fiber. The resultant plasmid provides expression of the fiber gene as described for pCLF and E4 function as described for pE4/Hygro.

A schematic plasmid map of pE4/Fiber, having 10610 bp, is shown in Figure 9. The complete nucleotide sequence of pE4/Fiber is listed in SEQ ID NO: 16 where the nucleotide position 1 corresponds to approximately 14 bp from the 3' end of the parent vector pCDNA3 backbone sequence. The 5' and 3 ends of the Ad5 E4 gene are located at respective nucleotide positions 21 and 3149 followed by fused BglII/BamHI sites and pCDNA3 backbone including the CMV promoter again followed by BglII/BamHI sites. The adenovirus leader sequence begins at nucleotide position 4051 and extends to 4366 followed by fused BamHI/BglII sites and the 5' and 3' ends of the fiber gene located at respective nucleotide positions 4372 and 6124.

Stable chromosonal insertions of pE4/Fiber in host cells are obtained as described above.

Example 2

Preparation of Adenoviral Gene Delivery Vectors Using Adenoviral Packaging Cell Lines

Adenoviral delivery vectors of this invention are prepared to separately lack the combinations of E1/fiber and E4/fiber. Such vectors are more replication-defective than those previously in use due to the absence of multiple viral genes. A preferred adenoviral delivery vector of this invention that is replication competent but only via a non-fiber means is one that only lacks the fiber gene but contains the remaining functional adenoviral regulatory and structural genes. Furthermore, the adenovirus delivery vectors of this invention have a higher capacity for insertion of foreign DNA.

A. Preparation of Adenoviral Gene Delivery Vectors Having Specific Gene Deletions and Methods of Use

To construct the E1/ fiber deleted viral vector containing the LacZ reporter gene construct, two new plasmids were constructed. The plasmid pΔ E1Bβgal was constructed as follows. A DNA fragment containing the SV40 regulatory sequences and *E. coli* β-galactosidase gene was isolated from pSVβgal (Promega) by digesting with VspI, filling the overhanging ends by treatment with Klenow fragment of DNA polymerase I in the presence of dNTP's and digesting with Bam H1. The resulting fragment was cloned into the EcoRV and BamHI sites in the polylinker of pΔ E1sp1B (Microbix Biosystems, Hamilton, Ontario) to form pΔE1Bβgal that therefore contained the left end of the adenovirus genome with the Ela region replaced by the LacZ cassette (nucleotides 6690 to 4151) of pSVβgal. Plasmid DNA may be prepared by the alkaline lysis method as described by Birnboim and Doly, *Nuc. Acids Res.*, *7*:1513-1523 (1978) or by the Quiagen method according to the manufacturer's instruction, from transformed cells used to expand the plasmid DNA was then purified by CsCl-ethidium bromide density gradient centrifugation. Alternatively, plasmid DNAs may be purified from *E. coli* by standard methods known in the art (e.g. *see* Sambrook *et al.*)

The second plasmid (pDV44), prepared as described herein, is derived from pBHG10, a vector prepared as described by Bett *et al.*, *Proc. Natl. Acad. Sci., USA*, 91:8802-8806 (1994), now described in International Application Publication Number WO 9500655, with methodology well known to one of ordinary skill in the art and also is commercially available from Microbix, which contains an Ad5 genome with the packaging signals at the left end deleted and the E3 region (nucleotides 28133:30818) replaced by a linker with a unique site for the restriction enzyme PacI. An 11.9 kb BamHI fragment, which contains the right end of the adenovirus genome, is isolated from pBHG10 and cloned into the BamHI site of pBS/SK(+) to create plasmid p11.3 having approximately 14,658 bp. A schematic of the plasmid map is shown in Figure 13. The p11.3 plasmid was then digested with PacI and SalI to remove the fiber, E4, and inverted terminal repeat (ITR) sequences.

This fragment was replaced with a 3,4 kb fragment containing the ITR segments and the E4 gene which was generated by PCR amplification from pBHG10 using the following oligonucleotide sequences (5' TGTACACCG GATCCGGCGCACACC3' SEQ ID NO: 17) and (5'CACAACGAGCTC

AATTAATTAATTGCCACATCCTC3' SEQ ID NO: 18). These primers incorporated sites for PacI and BamHI. Cloning this fragment into the PacI and blunt ended SalI sites of the p11.3 backbone resulted in a substitution of the fused ITRs, E4 region and fiber gene present in pBHG10, by the ITRs and E4 region alone. The resultant p11.3 plasmid containing the ITR and E4 regions, now called plasmid pDV43a, was then digested with BamHI. This BamHI fragment was then used to replace a BamHI fragment in pBHG10 thereby creating pDV44 in a pBHG10 backbone.

In an alternative approach to preparing pDV44 with an additional subcloning step to facilitate the incorporation of restriction cloning sites, the following cloning procedure was performed. pDV44 as above was constructed by removing the fiber gene and some of the residual E3 sequences from pBHG10 (Microbix Biosystems). As above, to simplify manipulations, the 11.9 kb BamHI fragment including the rightmost part of the Ad5 genome was removed from pBHG10 and inserted into pBS/SK. The resulting plasmid was termed p11.3. The 3.4 kb DNA fragment corresponding to the E4 region and both ITRs of adenovirus type 5 was amplified as described above from pBHG10 using the oligonucleotides listed above and subcloned into the vector pCR2.1 (Invitrogen) to create pDV42. This step is the additional cloning step to facilitate the incorporation of a SalI restriction site. pDV42 was then digested with PacI, which cuts at a unique site (bold type) in one of the PCR primers, and with SalI, which cuts at a unique site in the pCR2.1 polylinker. This fragment was used to replace the corresponding PacI/XhoI fragment of p11.3 (the pBS polylinker adjacent to the Ad DNA fragment contains a unique XhoI site), creating pDV43. Finally, pDV44 was constructed by replacing the 11.9 kb BamHI fragment of pBHG10 by the analogous BamHI fragment of pDV43. As generated in the first procedure, pDV44 therefore differs from pBHG10 by the deletion of Ad5 nucleotides 30819:32743 (residual E3 sequences and all but the 3'-most 41 nucleotides of the fiber open reading frame).

Thus, to summarize, the cloning procedures described above result in the production of a fiber-deleted Ad5 genomic plasmid (pDV44) that was constructed by removing the fiber gene and some of the residual E3 sequences from pBHG10 (Figure 16A). pDV44 contains a wild-type E4 region, but only the last 41 nucleotides of the fiber ORF (this sequence was retained to avoid affecting expression of the adjacent E4 transcription unit). Both pBHG10 and pDV44 contain unpackageable Ad5 genomes, and must be rescued by cotransfection and subsequent homologous recombination with DNA carrying functional packaging signals. In order to generate vectors marked with a reporter gene, either pDV44 or pBHG10 was cotransfected with pΔE1Bßgal, which contains the left end of the Ad5

genome with an SV40-driven ß-galactosidase reporter gene inserted in place of the E1 region.

In general, and as described below, the method for virus production by recombination of plasmids followed by complementation in cell culture involves the isolation of recombinant viruses by cotransfection of any one of the adenovirus packaging cell systems prepared in Example 1, namely 211A, 211B, 211R, A549, Vero cells, and the like, with plasmids carrying sequences corresponding to viral gene delivery vectors.

A selected cell line is plated in dishes and cotransfected with pDV44 and pΔE1Bßgal using the calcium phosphate method as described by Bett *et al.*, *Proc. Natl. Acad. Sci., USA, 91*:8802-8806 (1994). Recombination between the overlapping adenovirus sequences in the two plasmids leads to the creation of a full-length viral chromosome where pDV44 and pΔE1Bßgal recombine to form a recombinant adenovirus vector having multiple deletions. The deletion of E1 and of the fiber gene from the viral chromosome is compensated for by the sequences integrated into the packaging cell genome, and infectious virus particles are produced. The plaques thus generated are isolated and stocks of the recombinant virus are produced by standard methods.

A pDV44-derived virus is expected to be replication-defective due to the fiber deletion, so that the cells in which it is grown must complement this defect. The 211B cell line (a derivative of 293 cells which expresses the wild-type (wt) AD5 fiber and is equivalent to 211A on deposit with ATCC as described in Example 3) was used for rescue and propagation of the virus described here. pDV44 and pΔE1Bßgal were cotransfected into 211B cells, and the monolayers were observed for evidence of cytopathic effect (CPE). Briefly, for virus construction, cells were transfected with the indicated plasmids using the Gibco Calcium Phosphate Transfection system according to the manufacturer's instructions and observed daily for evidence of CPE.

One of a total of 58 transfected dishes showed evidence of spreading cell death at day 15. A crude freeze-thaw lysate was prepared from these cells and the resulting virus (termed Ad5.ßgal.ΔF) was plaque purified twice and then expanded. To prepare purified viral preparations, cells were infected with the indicated Ad and observed for completion of CPE. Briefly, at day zero, 211B cells were plated in DMEM plus 10% fetal calf serum at approximately 1 X 10^7 cells/150 cm^2 flask or equivalent density. At day one, the medium was replaced with one half the original volume of fresh DMEM containing the indicated Ad, in this case Ad5.ßgal.ΔF, at approximately 100 particles/cell. At day two, an equal volume of medium was

added to each flask and the cells were observed for CPE. Two to five days after infection, cells were collected and virus isolated by lysis via four rapid freeze-thaw cycles. Virus was then purified by centrifugation on preformed 15-40% CsCl gradients (111,000 x g for three hours at 4°C). The bands were harvested, dialyzed into storage buffer (10 mM Tris-pH 8.1, 0.9% NaCl, and 10% glycerol), aliquoted and stored at - 70°C. Purified Ad5.ßgal.ΔF virus particles containing human adenovirus Ad5.ßgal. ΔFgenome (described further below) have been deposited with the ATCC on January 15, 1999 as further described in Example 3.

For viral titering, as necessary in the below Examples, Ad preparations were titered by plaque assay on 211B cells. Cells were plated on polylysine-coated 6 well plates at 1.5 x 10^6 cells/well. Duplicate dilutions of virus stock were added to the plates in 1 ml/well of complete DMEM. After a five hour incubation at 37°C, virus was removed and the wells overlaid with 2 ml of 0.6% low-melting agarose in Medium 199 (Gibco). An additional 1 ml of overlay was added at five day intervals.

As a control, the first-generation virus Ad5.ß gal.wt, which is identical to Ad5.ßgal. ΔF except for the fiber deletion, was constructed by cotransfection of pBHG10 and pΔE1Bßgal (Figure 16B). In contrast to the low efficiency of recovery of the fiberless genome (1/58 dishes), all of 9 dishes cotransfected with pΔE1Bßgal and pBHG10 produced virus.

In a preferred embodiment of this invention as more fully described herein and below, a delivery plasmid is prepared that does not require the above-described recombination events to prepare a viral vector having a fiber gene deletion. In one embodiment, a single delivery plasmid containing all the adenoviral genome necessary for packaging but lacking the fiber gene is prepared from plasmid pFG140 containing full-length Ad5 that is commercially available from Microbix. The resultant delivery plasmid referred to as pFG140-f is then used with pCLF stably integrated cells as described above to prepare a viral vector lacking fiber. In a preferred aspect of this invention, the fiber gene is replaced with a therapeutic gene of interest for preparing a therapeutic delivery adenoviral vector. Other embodiments including production of fiberless vector with a complete TPL are described in Example 5.

Vectors for the delivery of any desired gene and preferably a therapeutic gene are prepared by cloning the gene of interest into the multiple cloning sites in the polylinker of commercially available pΔE1sp1B (Microbix Biosystems), in an analogous manner as performed for preparing p E1Bβgal as described above. The same cotransfection and recombination procedure is then followed as described herein to obtain viral gene delivery vectors as further discussed in later Examples.

1. Characterization of the Ad5.βgal.ΔF Genome

To confirm that the vector genomes had the expected structures and that the fiber gene was absent from the Ad5.ßgal.ΔF chromosome, the DNA isolated from viral particles was analyzed. Briefly, purified viral DNA was obtained by adding 10 µl of 10 mg/ml proteinase K, 40 µl of 0.5 M EDTA and 50 µl of 10% SDS to 800 µl of adenovirus-containing culture supernatant. The suspension was then incubated at 55C for 60 minutes. The solution was then extracted once with 400 µl of a 24:1 mixture of chloroform:isoamyl alcohol. The aqueous phase was then removed and precipitated with sodium acetate/ethanol. The pellet was washed once with 70% ethanol and lightly dried. The pellet was then suspended in 40 µl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Genomic DNA from both Ad5.ßgal.wt and Ad5.βgal.ΔF produced the expected restriction patterns (Figure 17A) following digestion with either EcoRI (Figure 17B) or with NdeI (data not shown). Southern blotting, performed with standard methods, with labeled fiber DNA as a probe demonstrated the presence of fiber sequence in Ad5.ßgal.wt but not in Ad5.ßgal. ΔF DNA (Figure 17C). As a positive control, the blot was stripped and reprobed with labeled E4 sequence. Fiber and E4 sequences were detected by using labeled inserts from pCLF and pE4/Hygro, respectively. As expected, E4 signal was readily detectable in both genomes at equal intensities (Figure 17C).

The complete nucleotide sequence of Ad5.βgal.ΔF is presented in SEQ ID NO: 27 and is contained in the virus particle on deposit with ATCC.

2. Characterization of the Fiberless Adenovirus Ad5.βgal.ΔF

To verify that Ad5.ßgal.ΔF was fiber-defective, 293 cells (which are permissive for growth of E1-deleted Ad vectors but do not express fiber) were infected with Ad5.ßgal. ΔF or with Ad5.ßgal.wt. Twenty-four hours post infection, the cells were stained with polyclonal antibodies directed either against fiber or against the penton base protein. Cells infected with either virus were stained by the anti-penton base antibody, while only cells infected with the Ad5.ßgal.wt control virus reacted with the anti-fiber antibody. This confirms that the fiber-deleted Ad mutant does not direct the synthesis of fiber protein.

3. Growth of the Fiber-Deleted Ad5.βgal.ΔF Vector in Complementing Cells

Ad5.βgal.ΔF was found to readily be propagated in 211B cells. As assayed by protein concentration, CsCl-purified stocks of either Ad5.βgal.ΔF or Ad5.ßgal.wt contained similar numbers of viral particles (Table 1), and the particles appeared to band normally on CsCl gradients. However, infectivity of the Ad5.βgal.ΔF particles was lower than the Ad5.ßgal.wt control, as indicated by an increased particle/PFU ratio (Table 1). This is likely due to a reduced amount of fiber protein incorporated into mutant particles during growth in the

Virus	CsCl-purified prepn	Cell line	Particles/ml[a]	PFU/ml[b]	Particle /PFU ratio	Fiber source
Ad5.βgal.wt	1	211 B	7.4×10^{11}	7.5×10^{10}	10	Ad chromosome
	2	211 B	3.0×10^{11}	5.0×10^{9}	60	Ad chromosome
Ad5.βgal.ΔF	3	211 B	7.7×10^{11}	3.5×10^{8}	2200	Packaging cells
	4	211 B	1.9×10^{12}	2.3×10^{9}	808	Packaging cells
	5	293	4.5×10^{11}	9.5×10^{6}	47400	None
	6	293	3.4×10^{11}	3.5×10^{7}	9700	None

[a]Calculated from viral protein concentration (1ug of protein = 4×10^9 particles).
[b]Assayed by plaquing on 211B cells.

Table 3

*Particle numbers and infectious titers of representative adenovirus preps. Each line represents a single CsCl-purified preparation of the indicated virus. Particle numbers were calculated from viral protein concentration (1 µg protein = 4×10^9 particles). Pfu was assayed by plaquing on 211B cells (see above).

211B cells (see below). Ad5.βgal.ΔF was also found to plaque more slowly than the control virus. When plated on 211B cells, Ad5.ßgal.wt plaques appeared within 5-7 days, while plaques of Ad5.βgal.ΔF continued to appear until as much as 15-18 days post infection. Despite their slower formation, the morphology of Ad5.βgal.ΔF plaques was essentially normal.

4. Production of Fiberless Ad5.ßgal. ΔF Particles

As Ad5.βgal.ΔF represents a true fiber null mutation and its stocks are free of helper virus, the fiber mutant phenotype was readily investigated. A single round of growth in cells (such as 293) which do not produce fiber generating a homogeneous preparation of fiberless Ad allowed for the determination of whether such particles would be stable and/or infectious. Either Ad5.ßgal.wt or Ad5.βgal.ΔF was grown in 293 or 211B cells, and the resulting particles purified on CsCl gradients as previously described. Ad5.βgal.ΔF particles were readily produced in 293 cells at approximately the same level as the control virus and behaved similarly on the gradients, indicating that there was not a gross defect in morphogenesis of fiberless capsids (Table 1).

As shown in Figure 18, particles of either virus contained similar amounts of penton base regardless of the cell type in which they were grown. This demonstrated that fiber is not required for assembly of the penton base complex into virions. However, as predicted, the Ad5.βgal.ΔF particles produced in 293 cells did not contain fiber protein. 211B-grown Ad5.βgal.ΔF also contained less fiber than the Ad5.ßgal.wt control virus (Figure 18). Importantly, the infectivities of the different viral preparations on epithelial cells (Table 1) correlated with the amount of fiber protein present. The fiberless Ad particles were several thousand-fold less infectious than the first-generation vector control on a per-particle basis, while infectivity of 211B-grown Ad5.βgal.ΔF was only 50-100 fold less than that of Ad5.ßgal.wt. These studies confirmed fiber's crucial role in infection of epithelial cells via CAR binding.

5. Composition and Structure of the Fiberless Ad5.ßgal. ΔF Particles

The proteins contained in particles of 293-grown Ad5.βgal.ΔF were compared to those in Ad5.ßgal.wt, to determine whether proteolysis or particle assembly was defective in this fiber null mutant (data not shown). The overall pattern of proteins in the fiberless particles was observed to be quite similar to that of a first-generation vector, with the exception of reduced intensity of the composite band resulting from both proteins IIIa and IV (fiber) (data not shown). The fiberless particles also had a reduced level of protein VII. Although substantial amounts of uncleaved precursors

to proteins VI, VII, and VIII were not seen, it is possible that the low-molecular weight bands migrating ahead of protein VII represent either aberrantly cleaved viral proteins or their breakdown products.

Cryo-electron microscopy was used to more closely examine the structure of the 293 grown Ad5.βgal.ΔF and of Ad5ßgal.wt. The fiber, which consists of an extended stalk with a knob at the end, was faintly visible in favorable orientations of wild-type Ad5 particles, but not in images of the fiberless particles (data not shown). Filamentous material likely corresponding to free viral DNA was seen in micrographs of fiberless particles. This material was also present in micrographs of the first-generation control virus, albeit at much lower levels.

Three-dimensional image reconstructions of fiberless and wild-type particles at ~20 Å resolution showed similar sizes and overall features, with the exception that fiberless particles lacked density corresponding to the fiber protein. The densities corresponding to other capsid proteins, including penton base and proteins IIIa, VI, and IX, were comparable in the two structures. This confirms that absence of fiber does not prevent assembly of these components into virions. The fiber was truncated in the wild-type structure as only the lower portion of its flexible shaft follows icosahedral symmetry. The RGD protrusions on the fiberless penton base were angled slightly inward relative to those of the wild-type structure. Another difference between the two penton base proteins was that there is a ~30 Å diameter depression in the fiberless penton base around the five-fold axis where the fiber would normally sit. The Ad5 reconstructions confirm that capsid assembly, including addition of penton base to the vertices, is able to proceed in the complete absence of fiber.

6. Integrin-Dependent Infectivity of Fiberless Ad5.ßgal. ΔF Particles

While attachment via the viral fiber protein is a critical step in the infection of epithelial cells, an alternative pathway for infection of certain hematopoietic cells has been described. In this case, penton base mediates both binding to the cells (via ß2 integrins) and internalization (through interaction with αv integrins). Particles lacking fiber might therefore be expected to be competent for infection of these cells, even though on a per-particle basis they are several thousand-fold less infectious than normal Ad vectors on epithelial cells.

To investigate this, THP-1 monocytic cells were infected with Ad5.ßgal.wt or with Ad5.βgal.ΔF grown in the absence of fiber. Infection of THP-1 cells was assayed by infecting 2 x 10^5 cells at the indicated m.o.i. in 0.5 ml of complete RPMI. Forty-eight hours post-infection, the cells were fixed with glutaraldehyde and stained with X-gal, and the percentage of stained cells was determined by light microscopy.

The results of the infection assay showed that the fiberless particles were only a few-fold less infectious than first-generation Ad on THP-1 cells (Figure 19A). In contrast to this, very large differences were seen in plaquing efficiency on epithelial (211B) cells (Table 1). Infection of THP-1 cells by either Ad5.βgal.ΔF or Ad5.ßgal.wt was not blocked by an excess of soluble recombinant fiber protein, but could be inhibited by the addition of recombinant penton base (Figure 19B). These results indicate that the fiberless Ad particles use a fiber-independent pathway to infect these cells. Furthermore, the lack of fiber protein did not prevent Ad5.βgal.ΔF from internalizing into the cells and delivering its genome to the nucleus, demonstrating that fiberless particles are properly assembled and are capable of uncoating.

The foregoing results with the recombinant viruses thus produced indicates that they can be used as gene delivery tools both in cultured cells and *in vivo* as described more fully in the Examples. For example, for studies of the effectiveness and relative immunogenicity of multiply-deleted vectors, virus particles are produced by growth in the packaging lines described in Example 1 and are purified by CsCl gradient centrifugation. Following titering, virus particles are administered to mice via systemic or local injection or by aerosol delivery to lung. The LacZ reporter gene allows the number and type of cells which are successfully transduced to be evaluated. The duration of transgene expression is evaluated in order to determine the long-term effectiveness of treatment with multiply-deleted recombinant adenoviruses relative to the standard technologies which have been used in clinical trials to date. The immune response to the improved vectors described here is determined by assessing parameters such as inflammation, production of cytotoxic T lymphocytes directed against the vector, and the nature and magnitude of the antibody response directed against viral proteins.

Versions of the vectors which contain therapeutic genes such as CFTR for treatment of cystic fibrosis or tumor suppressor genes for cancer treatment are evaluated in the animal system for safety and efficiency of gene transfer and expression. Following this evaluation, they are used as experimental therapeutic agents in human clinical trials.

B. Retargeting of Adenoviral Gene Delivery Vectors by Producing Viral Particles Containing Different or Altered Fiber Proteins

As the specificity of adenovirus binding to target cells is largely determined by the fiber protein, viral particles that incorporate modified fiber proteins or fiber proteins from different adenoviral serotypes (pseudotyped vectors) have different specificities. Thus, the methods of expression of the native Ad5 fiber protein in adenovirus packaging cells as described above is also applicable to production of different fiber proteins.

In one aspect of invention, chimeric fiber proteins are produced according to the methods of Stevenson *et al.*, *J. Virol., 69*:2850-2857 (1995). The authors showed that the determinants for fiber receptor binding activity are located in the head domain of the fiber and that isolated head domain is capable of trimerization and binding to cellular receptors. The head domains of adenovirus type 3 (Ad3) and Ad5 were exchanged in order to produce chimeric fiber proteins. Similar constructs for encoding chimeric fiber proteins for use in the methods of this invention are contemplated. Thus, instead of the using the intact Ad5 fiber-encoding construct prepared in Example 1 as a complementing viral vector in adenoviral packaging cells, the constructs described herein are used to transfect cells along with E4 and/or E1-encoding constructs.

Briefly, full-length Ad5 and Ad3 fiber genes were amplified from purified adenovirus genomic DNA as a template. The Ad5 and Ad3 nucleotides sequences are available with the respective GenBank Accession Numbers M18369 and M12411. Oligonucleotide primers are designed to amplify the entire coding sequence of the full-length fiber genes, starting from the start codon, ATG, and ending with the termination codon TAA. For cloning purposes, the 5' and 3' primers contain the respective restriction sites BamHI and NotI for cloning into pcDNA plasmid as described in Example 1A. PCR is performed as described above.

The resultant products are then used to construct chimeric fiber constructs by PCR gene overlap extension, as described by Horton *et al.*, *BioTechniques, 8*:525-535 (1990). The Ad5 fiber tail and shaft regions (5TS; the nucleotide region encoding amino acid residue positions 1 to 403) are connected to the Ad3 fiber head region (3H; the nucleotide region encoding amino acid residue positions 136 to 319) to form the 5TS3H fiber chimera. Conversely, the Ad3 fiber tail and shaft regions (3TS; the nucleotide region encoding amino acid residues positions 1 to 135) are

connected to the Ad5 fiber head region (5H; the nucleotide region encoding the amino acid residue positions 404 to 581) to form the 3TS5H fiber chimera. The fusions are made at the conserved TLWT (SEQ ID NO: 19) sequence at the fiber shaft-head junction.

The resultant chimeric fiber PCR products are then digested with BamHI and NotI for separate directional ligation into a similarly digested pcDNA 3.1. The TPL sequence is then subcloned into the BamHI as described in Example 1A for preparing an expression vector for subsequent transfection into 211 cells as described above or into the alternative packaging cell systems as previously described. The resultant chimeric fiber construct-containing adenoviral packaging cell lines are then used to complement adenoviral delivery vectors as previously described. Other fiber chimeric constructs are obtained using a similar approach with the various adenovirus serotypes known.

In an alternative embodiment, the methods of this invention contemplate the use of the modified proteins including novel epitopes as described by Michael *et al.*, *Gene Therapy, 2*:660-668 (1995) and in International Publication WO 95/26412. Both publications describe the construction of a cell-type specific therapeutic viral vector having a new binding specificity incorporated into the virus concurrent with the destruction of the endogenous viral binding specificity. In particular, the authors described the production of an adenoviral vector encoding a gastrin releasing peptide (GRP) at the 3' end of the coding sequence of the Ad5 fiber gene. The resulting fiber-GRP fusion protein was expressed and shown to assemble functional fiber trimers that were correctly transported to the nucleus of HeLa cells following synthesis.

Based on the teachings in the paper and International Publication, similar constructs are contemplated for use in the complementing adenoviral packaging cell systems of this invention for generating new adenoviral gene delivery vectors that are targetable, replication-deficient and less immunogenic. Heterologous ligands contemplated for use herein to redirect fiber specificity range from as few as 10 amino acids in size to large globular structures, some of which necessitate the addition of a spacer region so as to reduce or preclude steric hindrance of the heterologous ligand with the fiber or prevent trimerization of the fiber protein. The ligands are inserted at the end or within the linker region. Preferred ligands include those that target specific cell receptors or those that are used for coupling to other moieties such as biotin and avidin.

A preferred spacer includes a short 12 amino acid peptide linker composed of a series of serines and alanine flanked by a proline residue at each end. One of ordinary skill in the art is familiar with the preparation of linkers to accomplish sufficient protein presentation and for altering the binding specificity of the fiber protein without compromising the cellular events that follow viral internalization. Moreover, within the context of this invention, preparation of modified fibers having ligands positioned internally within the fiber protein and at the carboxy terminus as described below are contemplated for use with the methods described herein.

The preparation of a fiber having a heterologous binding ligand is prepared essentially as described in the above-cited paper. Briefly, for the ligand of choice, site-directed mutagenesis is used to insert the coding sequence for a linker into the 3' end of the Ad5 fiber construct in pCLF as prepared in Example 1.

The 3' or antisense or mutagenic oligonucleotide encodes a preferred linker sequence of ProSerAlaSerAlaSerAlaSerAlaProGlySer (SEQ ID NO: 20) followed by a unique restriction site and two stop codons, respectively, to allow the insertion of a coding sequence for a selected heterologous ligand and to ensure proper translation termination. Flanking this linker sequence, the mutagenic oligonucloetide contains sequences that overlap with the vector sequence and allo its incorporation into the construct. Following mutagenesis of the pCLF sequence adding the linker and stop codon sequences, a nucleotide sequence encoding a preselected ligand is obtained, linkers corresponding to the unique restriction site in the modified construct are attached and then the sequence is cloned into linearized corresponding restriction site. The resultant fiber-ligand construct is then used to transfect 211 or the alternative cell packaging systems previously described to produce complementing viral vector packaging systems for use with the methods of this invention.

In a further embodiment, intact fiber genes from different Ad serotypes are expressed by 211 cells or an alternative packaging system as previously described. A gene encoding the fiber protein of interest is first cloned to create a plasmid analogous to pCLF, and stable cell lines producing the fiber protein are generated as described above for Ad5 fiber. The adenovirus vector described which lacks the fiber gene is then propagated in the cell line producing the fiber protein relevant for the purpose at hand. As the only fiber gene present is the one in the packaging cells, the adenoviruses produced contain only the fiber protein of interest and therefore have the binding specificity conferred by the complementing protein. Such viral particles are used in studies such as those described above to determine their properties in experimental animal systems.

Example 3
Deposit of Materials

The following cell lines and plasmids have been deposited on September 25, 1996, with the American Type Culture Collection, 10801 University Blvd, Manassas, Virginia, USA (ATCC) under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty):Plasmid pE4/Hygro (accession number 97739), Plasmid pCLF (accession number 97737), 211 Cell Line (accession number CRL-12193) and 211A Cell Line (accession number CRL-12194)

The following virus, Ad5.βgal.ΔF , deposit was deposited on January 15, 1999, with the ATCC as listed above and provided with accession number VR2636.

Additionally, plasmids pDV60, pDV67, pDV69, pDV80 and pDV90 were also deposited at the ATCC on 5 January, 2000 and provided with accession numbers PTA-1144, PTA-1145, PTA-1146, PTA-1147 and PTA-1148 respectively.

Example 4
Complementation of Fiber-Defective and Fiber-Modified Virus

The native fiber protein is a homotrimer (Henry L.J. *et al., J. Virol. 68:*5239-5246 (1994)), and trimerization is essential for assembly of the penton/fiber complex (Novelli A *et al., J. Biol. Chem. 266:*9299-9303 (1991)). To assess the multimeric structure of the recombinant fiber protein produced by the cell lines, cells were labeled with 50 µCi/ml [^{35}S] Translabel (ICN) for two hours at 37°C, lysed in RIPA buffer, and fiber protein was immunoprecipitated as described (Harlow E et a., Antibodies. Cold Spring Harbour Laboratory, Cold Spring Harbor (1988). Immune complexes were collected on Protein A-Sepharose beads (Pierce), extensively washed with RIPA buffer, and incubated at room temperature in 0.1 M triethylamine, pH 11.5 to release bound fiber protein. A portion of the precipitated fiber was electrophoresed on a 8% SDS-PAGE gel under denaturing (1% SDS in loading buffer, samples boiled for 5 minutes) or semi-native (0.1% SDS in loading buffer, samples not heated) conditions.

As seen in Fig. 13, lines 211A, 211B, and 211R, but not the control 293 cells, expressed an immunologically reactive protein which migrated at the predicted molecular weight for trimer (186 kD) under seminative conditions and for monomer

(62 kD) under denaturing conditions. The behavior of the precipitated fiber was indistinguishable from that of purified baculovirus-produced recombinant Ad2 fiber (Wickham T *et al.*, *Cell 73*:309-319 (1993)) (the 58 kD Ad2 and 62 kD Ad5 fibers have very similar mobilities under these conditions).

To determine whether the fiber-expressing lines could support the growth of a fiber-defective adenovirus, we performed one-step growth experiments using the temperature-sensitive fiber mutant Ad H5*ts*142 (the gift of Harold Ginsberg). At the restrictive temperature (39.5°C), this mutant produces an underglycoslyated fiber protein which is not incorporated into mature virions (Chee-Sheung C. C *et al.*, *J. Virol 42*: 932-950 (1982)). This results in the accumulation of non-infectious viral particles. We asked whether the recombinant fiber protein expressed by our cell lines could complement the H5*ts*142 defect and rescue viral growth.

Cell lines 293, 211A, 211B and 211R (2×10^6 cells/sample) were infected with H5*ts*142 at 10 pfu/cell. 48 hours later, cells were detached with 25 mM EDTA and virus was harvested by four rapid freeze-thaw cycles. Debris was removed by a 10 minute spin at 1500 x g, and viral titers determined by fluorescent focus assay (Thiel J.F *et al.*, *Proc. Soc. Exp. Biol. Med. 125*:892-895 (1967)) on SW480 cells with a polyclonal anti-penton base Ab (Wickham T *et al.*, *Cell 73*:309-319 (1993)). As shown in Fig. 14, the fiber mutant virus replicated to high titers in 293 cells at 32.5°C (the permissive temperature), but to a much lower extent at the restrictive temperature of 39.5°C. The fiber-producing packaging lines 211A, 211B, or 211R supported virus production at 39°C to levels within two- to three-fold of those seen at the permissive temperature in 293 cells, indicating that these cells provided partial complementation of the fiber defect.

Interestingly, virus yields from the fiber-producing cell lines were also somewhat higher than those from 293 cells at 32.5°C (the 'permissive' temperature). This suggests that fiber produced by the ts142 virus may be partially defective even at the permissive temperature. Alternatively, a non-specific increase in adenoviral titer could result when viruses are grown in the packaging cells, by a mechanism not involving fiber complementation. However, it was found that viruses with wild type fiber genes (such as Ad.RSVβgal) replicate to identical levels either in our packaging lines or in 293 cells (data not shown). Taken together, these results demonstrate that the observed increase in H5*ts*142 growth is due to specific complementation of the fiber mutation.

Even in the fiber-expressing cell lines, the fiber mutant grows to higher titers at 32°C than at 39.5°C. This incomplete complementation may be due to the

packaging lines' expression of fiber at a level somewhat below that seen in a wild-type infection (data not shown). A recent study reported an E4-deleted vector which coincidentally reduced fiber protein expression, resulting in a large reduction in the titer of virus produced (Brough *et al., J. Virol. 70*:6497-6501 (1996)). Another possibility is that the defective *ts*142 fiber protein produced at the restrictive temperature might form complexes with some of the wild type protein produced by the cells and prevent its assembly into particles.

Although the fiber proteins of different Ad serotypes differ in the length of their shaft domains and in their receptor-binding knob domains, the N-terminal regions responsible for interaction with the viral penton base are highly conserved Arnberg N *et al., Virology 227*:239-244 (1997)) (Figure 15A). This suggests that fibers from many viral serotypes, with their different cell-binding specificities, may be amenable for use in producing gene delivery vectors.

In order to determine whether the recombinant Ad5 fiber produced by the packaging cells could be incorporated into particles of another adenovirus serotype, adenovirus type 3 was grown either in fiber-producing cell lines or in 293 cells. Viral particles were purified by two sequential centrifugations (3 h at 111,000 x g) on preformed 15-40% CsCl gradients to remove soluble cellular proteins and then dialyzed extensively against 10 mM Tris-HCl, pH 8.1, 150 mM NaCl, 10% glycerol. Ad5 fiber protein was detected by immunoblotting using the polyclonal anti-fiber serum, followed by detection with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Kirkegaard and Perry Laboratories) and the ECL chemiluminescence substrate (Amersham). The purified Ad3 particles contained Ad5 fiber protein after a single passage through a fiber-expressing cell line but not after passage through 293 cells
(Figure 15B). Previous work has demonstrated that Ad2 fiber is capable of interacting *in vitro* with Ad3 penton base (Fender *et al., Nature Biotech. 15*:52-56 (1997)), and our result demonstrates that the type 5 fiber protein produced by the cells is capable of assembling into complete Ad3 particles.

A vector based on Ad5 but containing the gene for the Ad7 fiber protein has been described (Gall J. *et al., J. Virol. 70*:2116-2123 (1996)), as well as Ads containing chimeric fiber genes (Krasnykh *et al., J. Virol. 70*:6839-6846 (1996) and Stevenson *et al., J. Virol. 69*:2850-2857 (1995)). Chimeric Ad5/Ad3 vectors have also been reported (Stevenson, S. *et al., J. Virol. 71*:4782-4790, (1997). Addition of a short peptide linker to the fiber in order to confer binding to a different cellular protein has also been reported (Michael *et al., Gene Therapy 2*:660-668 (1995). By

using packaging technology such as that presented here, Ad vectors equipped with different fiber proteins may be produced simply by growth in cells expressing the fiber of interest, without the time-consuming step of generating a new vector genome for each application.

Replacing or modifying the fiber gene in the vector chromosome would also require that the new fiber protein bind a receptor on the surface of the cells it which it is to be grown. The packaging cell approach will allow the generation of Ad particles containing a fiber which can no longer bind to its host cells, by a single round of growth in cells expressing the desired fiber gene. This will greatly expand the repertoire of fiber proteins which can be incorporated into particles, as well as simplifying the process of retargeting gene delivery vectors.

Finally, a novel fiber-independent pathway of infection has recently been described in hematopoietic cells, in which penton base provides the initial virus-cell interaction by binding to integrin $a_m b_2$ (Huang S. *et al., J. Virol 70:* 4502-4508 (1996)). This suggests that viral particles lacking fiber protein may be useful in targeting gene delivery to specific cell types via this pathway.

Example 5

Preparation of Alternative TPLs

The present invention contemplates the use of tripartite leader sequences (TPLs) that are useful in enhancing the expression of complementing adenoviral proteins, particularly fiber protein, for use in preparing an adenoviral gene delivery vector. One preferred TPL is the complete Ad5 tripartite leader contained in complementing vectors such as pDV67 and pDV69, both of which are prepared as described below. The complete Ad5 TPL was constructed by assembling PCR fragments. First, the third TPL exon (exon 3) (nt 9644-9731 of the Ad5 genome) was amplified from Ad5 genomic DNA using the synthetic oligonucleotide primers 5'CTCAACAATTGT**GGATCC**GTACTCC3'(SEQ ID NO: 28) and 5'GTGCTCAGC**AGATCT**TGCGACTGTG3' (SEQ ID NO: 29). The resulting product was cloned to the BamHI and BglII sites of pΔE1Sp1a (Microbix Biosystems) using novel sites in the primers (shown in bold) to create plasmid pDV52. A fragment corresponding to the first TPL exon (exon 1), the natural first intron (intron 1), and the second TPL exon (exon 2) (Ad5 nt 6049-7182) was then amplified using primers 5'GGCGCGTTC**GGATCC**ACTCTCTTCC3' (SEQ ID NO:30) and 5'CTA CATGCTAGGC**AGATCT**CGTTCGGAG3' (SEQ ID NO: 31), and cloned into the

BamHI site of pDV52 (again using novel sites in the primers) to create pDV55. This plasmid contains a 1.2 kb BamHI/BglII fragment consisting of the first TPL exon, the natural first intron, and the fused second and third TPL exons. The nucleotide sequence of the complete TPL containing the noted 5' and 3' restriction sites is shown in SEQ ID NO: 32 with the following nucleotide regions identified: 1-6 nt BamHI site; 7-47 nt first leader segment (exon 1); 48-1068 nt natural first intron (intron 1); 1069-1140 nt second leader segment (exon 2); 1141-1146 nt fused BamHI and BglII sites; 1147-1234 nt third leader segment (exon 3); and 1235-1240 nt BglII site.

TPLs fragments containing two of the three exons, exons in non-native order, or containing either the first or second TPL intron are also constructed for use in preparing complementing plasmids for use in the methods of the present invention. Briefly, DNA fragments containing any combination of 2 TPL exons can be constructed as follows: Exon 1 is amplified from genomic DNA as prepared above by using the oligonucleotides 5'GGCGCGTTC**GGATCC**ACTCTCTTCC3'(SEQ ID NO: 33) and 5'GGGAGT**AGATCT**CCCAACAG3' (SEQ ID NO: 34). Exon 2 is similarly amplified from the same genomic DNA using oligonucleotides 5'CCCTTTTTTTT**GGATCC**CTCGCGG3' (SEQ ID NO: 35) and 5'CTACATGCTAGGC**AGATCT**CGTTCGGAG3' (SEQ ID NO: 36). Exon 3 is amplified using the oligonucleotides 5'CTCAACAATTGTT**GGATCC**GTACTCC3' (SEQ ID NO: 37) and 5'GTGCTCAGC**AGATCT**TGCGACTGTG3' (SEQ ID NO: 38).

The amplified exons are ligated together in any desired number and/or order by virtue of the unique BamHI and BglII restriction sites (bold) in the primers for subsequent ligation into a construct analogous to pDV67, prepared as described below, for expression of viral structural genes.

Similarly, a fragment consisting of the first TPL exon (exon 1), the native first intron (intron 1), and the second TPL exon (exon 2) is produced by amplification from Ad5 genomic DNA with the oligonucleotide pair 5'GGCGCGTTC**GGATCC** ACTCTCTTCC3' (SEQ ID NO: 39) and 5'CTACATGCTAGGC**AGATCT** CGTTCGGAG3' (SEQ ID NO: 40). Finally, a fragment consisting of the second TPL exon (exon 2), the native second intron (intron 2), and the third TPL exon (exon 3) is produced by amplification using the oligonucleotides 5'CCCTTTTTTTT**GGATCC** CTCGCGG3' (SEQ ID NO: 41) and 5'GTGCTCAGC**AGATCT**TGCGACTGTG3' (SEQ ID NO: 42). Either of the intron-containing fragments are used either alone or in combination with another TPL fragment(s) in constructs analogous to pDV67. Introns in addition to adenoviral intron 1 used herein that have been shown to

increase the expression of recombinant proteins when included in expression constructs include SV40 VP1 intron, rabbit β-globin intron among others. The use of these alternative intron sequences are contemplated for use in preparing a TPL in the present invention.

Example 6
Preparation and Use of Adenoviral Packaging Cell Lines Containing Plasmids Containing Alternative TPLs

Plasmids were first constructed as described below that contained TPLs are described above. The resultant plasmids containing different selectable markers such as neomycin or zeocin were then used to prepare stable cell lines for use as complementing vectors for preparing adenoviral vectors for use in the present invention. In a preferred embodiment, the resulting cell lines represent improvements over preexisting fiber-complementing cell lines in that fiber expression is enhanced with the use of alternative TPLs.

A. pDV60

pDV60 was constructed by inserting this TPL cassette of SEQ ID NO: 32 into the BamHI site upstream of the Ad5 fiber gene in pcDNA3/Fiber, a neomycin selectable plasmid, prepared as described in Example 1 and also as described by Von Seggern *et al.*, *J. Gen Virol., 79*: 1461 (1998). The nucleotide sequence of pDV60 is listed in SEQ ID NO: 43.

B. pDV61

To construct pDV61, an Asp718/NotI fragment containing the CMV promoter, partial Ad5 TPL, wildtype Ad5 fiber gene, and bovine growth hormone terminator was transferred from pCLF, prepared as described in Example 1 and also as described by Von Seggern *et al.*, *J. Gen Virol., 79*: 1461 (1998), to a zeocin selectable cloning vector referred to as pCDNA3.1/Zeo (+) (commercially available from Invitrogen and the sequence is also available).

C. pDV67

In an analogous process, pDV67 containing complete TPL was constructed by transferring an Asp 718/Xbal fragment from pDV60 to the pcDNA3.1/Zeo(+) backbone. The nucleotide sequence of pDV67 is listed in SEQ ID NO: 44.

D. pDV69

To prepare pDV69 containing a modified fiber protein, the chimeric Ad3/Ad5 fiber gene was amplified from pGEM5TS3H (Stevenson *et al., J. Virol., 69:* 2850-2857, 1995)) using the primers 5'ATGGGAT CAAGATGAAGCGCGCAAGACCG3' (SEQ ID NO: 45) and 5'CACTATAGCGGCCGCATTCTCAGTCATCTT3' (SEQ ID NO: 46), and cloned to the BamHI and NotI sites of pcDNA3.1/Zeo(+) via novel BamHI and NotI sites engineered into the primers to create pDV68. Finally, the complete TPL fragment described above was then added to the unique BamH1 site of pDV68 to create pDV69. The nucleotide sequence of pDV69 is listed in SEQ ID NO: 47.

E. Preparation of Stable Adenovirus Packaging Cell Lines

E1-2a S8 cells are derivatives of the A549 lung carcinoma line (ATCC # CCL 185) with chromosomal insertions of the plasmids pGRE5-2.E1 (also referred to as GRE5-E1-SV40-Hygro construct and listed in SEQ ID NO: 48) and pMNeoE2a-3.1 (also referred to as MMTV-E2a-SV40-Neo construct and listed in SEQ ID NO: 49), which provide complementation of the adenoviral E1 and E2a functions, respectively. This line and its derivatives were grown in Richter's modified medium (BioWhitaker) + 10% FCS. E1-2a S8 cells were electroporated as previously described (Von Seggern *et al., J. Gen Virol., 79*: 1461 (1998)) with pDV61, pDV67, or with pDV69, and stable lines were selected with zeocin (600 µg/ml). The cell line generated with pDV61 is designated 601. The cell line generated with pDV67 is designated 633 while that generated with pDV69 is designated 644. Candidate clones were evaluated by immunofluorescent staining with a polyclonal antibody raised against the Ad2 fiber. Lines expressing the highest level of fiber protein were further characterized.

For the S8 cell complementing cell lines, to induce E1 expression, 0.3 µM of dexamethasone was added to cell cultures 16-24 hours prior to challenge with virus for optimal growth kinetics. For preparing viral plaques, 5 X 10^5 cells/well in 6 well plates are prepared and pre-induced with the same concentration of dexamethasone

the day prior to infection with 0.5 μM included at a final concentration in the agar overlay after infection.

F. Development of Cell Lines for Complementation of E1⁻/E2a⁻ Vectors

This example shows the construction of S.8 cells

The Adenovirus 5 genome was digested with ScaI enzyme, separated on an agarose gel, and the 6,095 bp fragment comprising the left end of the virus genome was isolated. The complete Adenovirus 5 genome is registered as Genbank accession #M73260, incorporated herein by reference, and the virus is available from the American Type Culture Collection, Manassas, Virginia, U.S.A., under accession number VR-5. The ScaI 6,095 bp fragment was digested further with ClaI at bp 917 and BglII at bp 3,328. The resulting 2,411 bp ClaI to BglII fragment was purified from an agarose gel and ligated into the superlinker shuttle plasmid pSE280 (Invitrogen, San Diego, CA), which was digested with ClaI and BglII, to form pSE280-E. (Figure 23).

Polymerase chain reaction (PCR) was performed to synthesize DNA encoding an XhoI and SalI restriction site contiguous with Adenovirus 5 DNA bp 552 through 924. The primers which were employed were as follows:

5' end, Ad5 bp 552-585:

5'-GTCACTCGAGGACTCGGTC-GACTGAAAATGAGACATATTATCTGCCACGGAC C-3' (SEQ ID NO: 66)

3' end, Ad5 bp 922-891:

5'-CGAGATCGATCACCTCCGGTACAAGGTTTGGCATAG-3' (SEQ ID NO: 67)

This amplified DNA fragment (sometimes hereinafter referred to as Fragment A) then was digested with XhoI and ClaI, which cleaves at the native ClaI site (bp 917), and ligated to the XhoI and ClaI sites of pSE280-E, thus reconstituting the 5(end of the E1 region beginning 8 bp upstream of the ATG codon.

PCR then was performed to amplify Adenovirus 5 DNA from bp 3,323 through 4,090 contiguous with an EcoRI restriction site. The primers which were employed were as follows:

5' end, Ad5 bp 3323-3360:

5'-CATGAAGATCTGGAAGGTGCTGAGGTACGATGAGACC-3' (SEQ ID NO: 68)

3' end, Ad5 bp 4090-4060:

5'-GCGACTTAAGCAGTCAGCTG-AGACAGCAAGACACTTGCTTGATCCAAATCC-3' (SEQ ID NO: 69)

This amplified DNA fragment (sometimes hereinafter referred to as Fragment B) was digested with BglII, thereby cutting at the Adenovirus 5 BglII site (bp 3,382) and EcoRI, and ligated to the BglII and EcoRI sites of pSE280-AE to reconstruct the complete E1a and E1b region from Adenovirus 5 bp 552 through 4,090. The resulting plasmid is referred to as pSE280-E1 (Figure 23).

A construct containing the intact E1a/b region under the control of the synthetic promoter GRE5 was prepared as follows. The intact E1a/b region was excised from pSE280-E1, which was modified previously to contain a BamHI site 3' to the E1 gene, by digesting with XhoI and BamHI. The XhoI to BamHI fragment containing the E1a/b fragment was cloned into the unique XhoI and BamHI sites of pGRE5-2/EBV (Figure 4, U.S. Biochemicals, Cleveland, Ohio) to form pGRE5-E1 (Figure 24).

Bacterial transformants containing the final construct were identified. Plasmid DNA was prepared and purified by banding in CsTFA prior to use for transfection of cells.

Construction of plasmid including Adenovirus 5 E2A sequence.

The Adenovirus 5 genome was digested with BamHI and SpeI, which cut at bp 21,562 and 27,080, respectively. Fragments were separated on an agarose gel and the 5,518 bp BamHI to SpeI fragment was isolated. The 5,518 bp BamHI to SpeI fragment was digested further with SmaI, which cuts at bp 23,912. The resulting 2,350 bp BamHI to SmaI fragment was purified from an agarose gel, and ligated into the superlinker shuttle plasmid pSE280, and digested with BamHI and SmaI to form pSE280-E2 BamHI-SmaI (Figure 26).

PCR then was performed to amplify Adenovirus 5 DNA from the SmaI site at bp 23,912 through 24,730 contiguous with NheI and EcoRI restriction sites. The primers which were employed were as follows:

5' end, Ad5 bp 24,732-24,708:

5'-CACGAATTCGTCAGCGCTTCTCGTCGCGTCCAAGACCC-3' (SEQ ID NO: 70)

3' end, Ad5 bp 23,912-23,934:

5'-CACCCCGGGGAGGCGGCGGCGACGGGGACGGG-3' (SEQ ID NO: 71)

This amplified DNA fragment was digested with SmaI and EcoRI, and ligated to the SmaI and EcoRI sites of pSE280-E2 Bam-Sma to reconstruct the complete E2a region from Ad5 bp 24,730 through 21,562. The resulting construct is pSE280-E2a. (Figure 27.)

In order to convert the BamHI site at the 3' end of E2a to a SalI site, the E2a region was excised from pSE280-E2a by cutting with BamHI and NheI, and recloned into the unique BamHI and NheI sites of pSE280. (Figure 6.) Subsequently, the E2a region was excised from this construction with NheI and SalI in order to clone into the NheI and SalI sites of the pMAMneo (Clonetech, Palo Alto, CA) multiple cloning site in a 5' to 3' orientation, respectively. The resulting construct is pMAMneo E2a. (Figure 27).

Bacterial transformants containing the final pMAMneo-E2a were identified. Plasmid DNA was prepared and purified by banding in CsTFA. Circular plasmid DNA was linearized at the XmnI site within the ampicillin resistance gene of pMAMneo-E2a, and further purified by the phenol/chloroform extraction and ethanol precipitation prior to use for transfection of cells.

Transfection and selection of cells.

In general, this process involved the sequential introduction, by calcium phosphate precipitation, or other means of DNA delivery, of two plasmid constructions each with a different viral gene, into a single tissue culture cell. The cells were transfected with a first construct and selected for expression of the associated drug resistance gene to establish stable integrants. Individual cell clones were established and assayed for function of the introduced viral gene. Appropriate candidate clones then were transfected with a second construct including a second viral gene and a second selectable marker. Transfected cells then were selected to establish stable integrants of the second construct, and cell clones were established. Cell clones were assayed for functional expression of both viral genes.

In order to determine the most suitable cell lines for the above-mentioned transfections, sequential transfections and selections were carried out with the following parental cell types:

A549 (ATCC Accession No. CCL-185);

Hep-2 (ATCC Accession No. CCL-23); or

KB (ATCC Accession No. CCL-17).

Appropriate selection conditions were established for both G418 and hygromycin B for all three cell lines by standard kill curve determination.

Transfection of cell lines with plasmids including E1 and E2a regions.

pMAMNeo-E2a was linearized with XmnI with the Amp^R gene, introduced into cells by transfection, and cells were selected for stable integration of this plasmid by G418 selection until drug resistant colonies arose. The clones were isolated and screened for E2a expression by staining for E2a protein with a polyclonal antiserum,

and visualizing by immunofluorescence. E2a function was screened by complementation of the temperature-sensitive mutant Ad5ts125 virus which contains a temperature-sensitive mutation in the E2a gene. (Van Der Vliet, et al., J. Virology, Vol. 15, pgs. 348-354 (1975)). Positive clones expressing the E2a gene were identified and used for transfection with the 7 kb EcoRV to XmnI fragment from pGRE5-E1 (Figure 5), which contains the GRE5 promoted E1a/b region plus the hygromycin^R gene. Cells were selected for hygromycin resistance and assayed for E1a/b expression by staining with a monoclonal antibody for the E1 protein (Oncogene Sciences, Uniondale, N.Y.). E1 function was assayed by ability to complement an E1-deleted vector. At this point, expression and function of E2a was verified as described above, thus establishing the expression of both E1a/b and E2a in the positive cell clones.

One of the transfected A549 cell lines showed good E1a/b and E2a expression and was selected for further characterization. It was designated the S8 cell line.

G. Preparation of Adenoviral Vectors Containing Ad5.βgal.ΔF Genome in S8 Improved Fiber-Complementing Cell Lines

To prepare adenoviral vectors containing Ad5.βgal.ΔF in S8 cells containing alternative forms of TPL for enhancing the expression of fiber proteins, the protocol as described in Example 2 for preparing Ad5.βgal.ΔF in 211B cells was followed with the exception of pretreatment with 0.3 μM dexamethasone for 24 hours as described above. Thus, viral particles with the wildtype Ad5 fiber protein on their surface and containing the fiberless Ad5.βgal.ΔF genome were produced in 633 cells. Particles produced in 644 cells also contained the fiberless Ad5.βgal.ΔF genome, but had the chimeric 5T3H fiber protein, with the Ad3 fiber knob, on their surface.

The preparation of the cell lines and demonstration of stable nuclear expression of either wild-type Ad5 fiber protein or chimeric Ad5/Ad3 protein is shown in Figure 20. In the figure, schematic diagrams are presented of the constructs used to generate the cell lines as well as immunofluorescence results indicating the presence of expressed fiber protein in the nucleus of the cells. An indirect immunofluorescence assay of A549 based cell lines which stably express the different Ad fibers is shown. Line 633 expresses the native Ad5 fiber protein and line 644 expresses a chimeric fiber protein with the tail and shaft domains of the Ad5

protein and the knob domain of the Ad3 fiber. Previous work (Stevenson et al., 1996) showed that a virus containing this protein had the tropism expected for Ad3.

Thus, these viral preparations, prepared as described herein and in Example 2, are useful for targeting delivery of Ad5.βgal.ΔF fiberless genome with either wild-type or modified fibers, embodiments of which uses have been previously discussed and as further exemplified with the pseudotyping and infectivity results described in Example 7.

Example 7

Pseudotyping and Infectivity of Recombinant Adenoviral Vectors Produced with Improved Fiber-Complementing Cell Lines

A. Pseudotyping of Ad5.βgal.ΔF

To verify that adenoviral vectors were produced had altered tropisms, viral particles were purified from either 633 (expressing wilt type Ad5 fiber) or 644 cells (expressing the chimeric Ad5/Ad3 fiber) 10μg of the purified particles were Western blotted and probed with a polyclonal rabbit antibody against the Ad2 fiber (which detects both the Ad5 and chimeric 5T3H fiber proteins.). Equal amounts of purified Ad.βgal.wt or Av9LacZ (which has the chimeric fiber gene in the viral chromosome) were run as controls. The results are shown in Figure 21 where both fiber proteins were detectable confirming pseudotyping.

B. Infectivity of Cells with 633 or 644 Generated Virus Particles

The cell lines, 633 or 644, prepared as described above, were infected with the indicated number of particles/cell of Ad5.βgal.ΔF and virus particles produced. Virus was then used to infect, as previously described, selected cell lines as shown in Figure 22, including 211B, MRC-5 human fibroblasts, A-10 rat aortic endothelial cells, and THP-1 human monocytic cells. Unbound virus was removed by washing the cells and the cells were further incubated at 37°C for 48 hours. Cells were then fixed with glutaraldehyde and stained with X-gal. The percentage of stained cells was then determined by light microscopy where all experiments were done in triplicate.

The results shown in Figure 22 indicate that adenoviral vectors could be retargeted by pseudotyping using packaging cell lines expressing different fiber

proteins. The data marked with "none" indicates virus grown in 293 cells and lacking fiber, while "Ad5" indicates virus prepared in 633 cells (containing the wild type fiber) and Ad3 indicates virus prepared in 644 cells (containing the chimeric 5T3H fiber.) Particles containing either fiber were equally infectious on 211B cells, while MRC-5 fibroblasts and THP-1 cells were more readily infected by virus containing the chimeric fiber. The A-10 rat endothelial cells were more readily infected by particles containing the wildtype Ad5 fiber protein.

Example 8

Targeted Gene Delivery Using Viral Vector Particles Lacking Fiber Protein

An alternative mode of entry for adenoviral infection of hematopoietic cells has been described by Huang, *et al.*, *J. Virol.*, *69*:2257-2263 (1995) which does not involve the fiber protein-host cell receptor interaction. As infection of most other cell types does require the presence of fiber protein, vector particles which lack fiber may preferentially infect hematopoietic cells, such as monocytes or macrophages.

To produce a fiber-free adenovirus vector particle, a vector lacking the fiber gene as described above in Example 2A but containing a gene of interest for delivery is amplified by growth in cells which do not produce a fiber protein, such as the 211 cells prepared in Example 1 or 293 or S8 cells as described herein, thereby producing large numbers of particles lacking fiber protein. The recovered fiber-free viral particles are then used to deliver the inserted gene of interest following the methods of this invention via targeting mechanisms provided by other regions of the adenoviral vector, *i.e.*, via the native penton base.

A. Construction of an Adenovirus Vector Deleted for E1, E3, and Fiber, and Carrying a Therapeutic Gene of Interest

A general method of constructing a fiber-deleted Ad vector containing a therapeutic gene of interest (in this example, the Herpes Simplex Virus Thymidine Kinase (TK) gene) is described here. Linear viral DNA is isolated from a preparation of Ad5.βgal.ΔF particles. This DNA is digested with the restriction enzyme ClaI, which removes the leftmost viral sequences including the left ITR, the

packaging signals, and part of the SV40-driven β-galactosidase gene. The large ClaI fragment with the remainder of the fiber-deleted viral genome is then isolated by centrifugation on a sodium chloride or sucrose gradient. The plasmid pAdShuttleTK, which contains the left part of the Ad chromosome with an RSV-driven TK gene inserted in place of the E1 region, is linearized by digestion with NotI. The nucleotide sequence of the pAdShuttleTK is shown in SEQ ID NO: 50. The large ClaI fragment of Ad5.βgal.ΔF and the linearized pAdShuttleTK are cotransfected into 211B cells, and an infectious adenovirus genome is generated by homologous recombination. A virus deleted for E1, E3, and fiber that contains the TK cassette in the place of the E1 deletion is thus recovered. A virus containing any desired therapeutic gene of interest can be created in this manner by replacing the TK gene of the example with the gene of interest.

An alternative method of constructing a fiber-deleted genome containing a therapeutic gene (in this example the *retinal degeneration-slow (RDS)* gene driven by the CMV immediate early promoter) is described here. RDS is a protein expressed in photoreceptors, and essential for their proper development and functioning. *RDS* mutations have been implicated in retinal degenerative disorders, and transfer of the wildtype *RDS* gene by means of an Ad vector provides an avenue towards treating such disorders.

A plasmid (pDV50) analogous to pΔE1Bβgal but containing a CMV-driven *RDS* gene was constructed as follows. First, a fragment containing the CMV promoter and enhancer was excised from pCHaMIEP by digestion with HindIII, filling the overhanging ends with the large fragment of *E. coli* DNA polymerase 1, ligation of BamHI linkers (5'CGCGGATCCCG3' SEQ ID NO: 51) to the blunt ends, and digesting with BamHI. The resulting fragment was then ligated into the BamHI site of pΔE1sp1a (Mikrobix) to create pDV45. A fragment containing the SV40 polyadenylation signal was amplified from pSVβgal (Promega) using the oligonucleotides 5'CTGACAAACTC**AGATCT**TGTTTATTG3' (SEQ ID NO: 51) and 5'GTCGACTCTAGA**GGATCC**AGA3' (SEQ ID NO: 52). This fragment was ligated into the BglII site of pDV45 to create pDV46, using the unique BamHI and BglII sites (bold type) in the primers. Finally, the human *RDS* open reading frame was amplified from the plasmid pRDS-T7 using the oligonucleotides 5'CCGGAC**TCT AGA**TGGCAACCATGGCGCTAC3' (SEQ ID NO: 53) and 5'GGA GGGG**AAGCTT**GGCCCTCAGCCAGCCTCT3' (SEQ ID NO: 54). This fragment was inserted into the HindIII and XbaI sites of pDV46, again using unique restriction sites in the primers, to create pDV50. pDV50 therefore contains a cassette

consisting of the CMV promoter, the *RDS* open reading frame, and the SV40 terminator sequences inserted in place of the Ad5 E1 region.

In a manner analogous to the construction of Ad5.βgal.ΔF , pDV50 and pDV44 are then co-transfected into 211B cells, and an infectious Ad genome (Ad5.RDS.ΔF) is recovered. A fiber-deleted Ad vector containing any desired gene to be expressed can be constructed by replacing the RDS gene of this example with the gene of interest.

Example 9

Transient Transcomplementation

Human adenovirus type 5 (Ad5) is being developed as a vector for gene therapy. Its ability to deliver therapeutic genes to cells is mediated by the interaction of the adenoviral fiber protein with the coxsackievirus-adenoviral receptor (CAR). Because a wide-range of cells express CAR, it can be difficult to use adenoviruses to deliver genes to specific cell types. One way to address this is to target the virus to a particular cell type by genetically altering the fiber. However, the genetic manipulations involved in cloning and production of the viruses with altered fibers can be time-consuming. Thus it would be a significant advancement in the field of adenoviral gene therapy to have a more streamlined system for testing modified fiber genes. An *in vitro* system has thus been developed that involves infection of tissue culture cells with a fiber-deleted Ad and transient co-transfection with a plasmid directing fiber expression. This system allows one to produce and evaluate such modified fibers in the context of a viral particle easily and quickly. In addition this system can be envisioned to actually produce therapeutic quantities of adenoviral vectors with modified fiber proteins, with such fibers having a new tropism added by insertion of a desired ligand into the fiber gene. These fibers may also have the natural tropism (*i.e.* binding to CAR) ablated.

Plasmids used were pDV60 and pDV55, prepared as described herein. pDV60 is an pcDNA3.1-based expression plasmid that contains the CMV promoter, Ad5 tripartite leader, an intron, and the Ad5 fiber gene sequence. pDV55 contains no fiber gene and serves as the negative control. Ad5.βgal.ΔF and 211B are described above. 293T cells are identical to 293 cells except they express an integrated SV40 large T antigen gene. HDF cells are human diploid fibroblasts. 293T cells express CAR and α_v integrins; HDF cells express α_v integrins but no CAR. Transfections with fiber expression plasmids were performed with

Lipofectamine (GIBCO-BRL) using 20mg DNA and 50ml Lipofectamine per 15cm dish. Cells were maintained in DMEM supplemented with 10% fetal bovine serum.

The fiber deletion mutation of Ad5.βgal.ΔF is complemented in *trans* by passaging virions through 211B, a cell line that stably expresses functional Ad5 fiber. The present system was designed to complement Ad5.βgal.ΔF by modified fibers expressed from transfected episomal plasmids in 293T cells. The result is a simplified and rapid method to incorporate modified fibers on a viral particle containing the Ad5.βgal.ΔF genome that does not require propagation of the virus.

The feasibility of transcomplementation of Ad5.βgal.ΔF with episomal fiber-expressing plasmids was demonstrated in the following experiment. 293T cells were transfected with one of two plasmids: pDV55, which expresses no fiber or pDV60, which expresses wildtype Ad5 fiber. Fiber expression persists for at least six days, suggesting that the plasmid is stable as an episome for this amount of time Twenty-four hours after transfection, these cells were infected at 2000 particles/cell with Ad5.βgal.ΔF passaged through 211B cells. Seventy-two hours later, a crude viral lysate (CVL) was generated by exposing the cells to five freeze-thaw cycles. Viral particles were purified by cesium chloride gradient centrifugation. The resulting virions incorporated the fiber expressed from the episomal plasmid, as confirmed by Western blots performed with an antibody specific to the Ad5 fiber.

To demonstrate the functionality of these virions, the transduction efficiency was tested. The virions containing no fiber (pDV55) or wildtype fiber (pDV60) were applied to monolayers of 293T and HDF cells at different multiplicity of infection (MOI's). 293T cells express CAR and a α_v'integrins; HDF cells express α_v integrins but no CAR. After 2 days, the cells were fixed and stained with X-gal to detect the βgalactosidase reporter gene activity. The results showed low transduction efficiency for the pDV55-complemented virions in both cell lines. As expected, the pDV60-complemented virions transduced 293T cells to a high degree but did not transduce HDF cells, indicating that functional fiber proteins had been expressed from the episomal plasmids and incorporated into the virions. This transduction efficiency was comparable to or better than that of Ad5.βgal.ΔF virions passaged through the 211B cells.

Episomal plasmid transcomplementation system is suitable for quickly expressing and evaluating the properties of modified fibers in the context of a viral particle. Episomal plasmid transcomplementation will also be of great utility for quickly evaluating a bank of modified fibers for other binding properties, including novel tropism and the ablation of the native tropism. In addition to the rapid

generation and testing of large numbers of modified fibers, there are other advantages to the Ad5.βgal.ΔF transcomplementation system in terms of production and safety. Episomal plasmid transcomplementation has the inherent advantage over transcomplementation in that it is not necessary to make a stable cell line for every modified fiber with which you want to complement Ad5.βgal.ΔF . Because the Ad5.βgal.ΔF is deleted in E1, E3 and fiber, there is an additional gene deletion compared to other first generation vectors. This makes Ad5.βgal.ΔF more replication defective and presumably safer. In addition, the presence of the fiber gene deletion decreases the opportunity to generate replication-competent virus via recombination in the packaging cells. In terms of production a single Ad vector prep could be retargeted to any number of different cell types simply by transfecting the cells with the appropriate fiber-expression construct.

Example 10

Adenoviral Gene Delivery Vectors Containing the Ad37 Fiber Protein

Adenovirus type 37 (subgroup D) has been associated with infections of the eye
and genital tract, and may be useful for targeting these tissues or other mucous membranes, as well as other cell types. The tropism of Ad37 is due to the binding preference of its fiber protein, which binds to an as yet-unidentified receptor located on the surface of cells including Chang C, conjunctival epithelial cell line (Huang *et al., J. Virology 73(4)*:2798-2802 (1999)). As this fiber directs viral infection to cell types different than those infected by Ad5, it is likely to provide a method for targeting gene delivery. This example describes construction of packaging cell lines expressing the Ad37 fiber protein, and their use in generating particles of a fiber-deleted Ad vector (such as Ad5.βgal.ΔF) containing this fiber protein. The fiber protein is attached to the viral capsid by binding to the penton base protein through its N-terminus, and the Ad37 fiber was modified in order to make its N-terminal sequence more closely match that of the Ad5 protein to ensure that it would efficiently bind the Ad5 penton base in these vectors.

1. Construction of an Expression Plasmid for the Ad37 Fiber Protein (pDV80)

This plasmid uses the same regulatory elements as contained in pDV60, pDV67, and pDV69 to express the Ad37 fiber in packaging lines, and was constructed in two steps. First, the Ad37 fiber open reading frame was amplified from Ad37 genomic DNA (obtained from the ATCC - accession number VR-929)using the synthetic oligonucleotides primers L37 (5' TGT CTT **GGA TCC** AAG *ATG* AAG CGC GCC CGC CCC AGC GAA GAT GAC TTC 3') (SEQ ID NO: 56) and 37FR (5' AAA CAC **GGC GGC CGC** TCT TTC ATT CTT G 3') (SEQ ID NO: 57). L37 contains nucleotides that differ from the Ad37 genomic sequence in order to add an unique *Bam* H1 site (bold in the above sequence) and create point mutations to make the N-terminal sequence of the fiber more closely match that of the Ad6 protein (underlined in the above sequence; the start codon is italicized). 37FR incorporates changes to create a unique *Not* 1 site (bold). The PCR product was inserted into the *Bam* H1 and *Not* 1 sites of pCDNA3.1zeo(+) (Invitrogen) to create pDV78. The correct sequence of the Ad37 fiber gene, including the predicted changes, was confirmed by sequencing.

Second, a 1.2 kb *Bam* H1/*Bgl* II fragment containing an adenovirus type 5 tripartite leader was excised from pDV55 (DVS 1999) and inserted into the *Bam* H1 site of pDV78 to create pDV80 (SEQ ID NO:64)

2. Isolation of Cell Lines Expressing the Ad37 Fiber Protein

pDV80 DNA was purified using the Qiagen method and electroporated into the adenovirus-complementing cell line E1-2a S8 (Gorziglia *et al., J. Virology 70(5)*:4173-4178 (1996)) as previously described (Von Seggern, *et al., J. Gen. Virol. 79*:1461-1418), and stable clones were selected with 600 µg/ml zeocin (Invitrogen). Clones were expanded and screened for fiber expression by indirect immunofluorescence using a rabbit polyclonal antibody directed against the Ad37 fiber. Two clones (lines 705 and 731) that expressed the protein at a uniformly high level were selected for further study.

3. Production of Pseudotyped Ad Vector Particles

To generate vector particles equipped ('pseudotyped') with the Ad37 fiber protein, the Ad37 fiber-expressing 705 cells were infected (approximately 1000 particles/cell) with Ad5.βgal.ΔF or with Ad5.GFP.ΔF.

Ad5.βgal.ΔF is prepared as previously described. Ad5.GFP.ΔF was constructed by recombination in bacteria using a modification of the method of (He, *et al., PNAS 95*:2509-2514 (1998)). First, a fiber-deleted genomic plasmid was constructed by removing the fiber gene from pAdEasy1 (He, *et al., PNAS 95*:2509-2514 (1998)). pDV43 (Von Seggern, *et al., J. Virol. 73*:1601-1608 (1999)) was digested with *Pac* 1, the ends blunted by treatment with the large fragment of *E. coli* DNA polymerase and dNTPs, and the product re-ligated. The resulting plasmid, pDV76, is identical to pDV43 except for loss of the *Pac* 1 site and contains the right end of the Ad5 genome with E3 and fiber deletions. A 4.2 kb fragment was amplified from pDV76 using the oligonucleotides primers 5' CGC GCT GAC TCT TA GG**A CTA** GTT TC 3' (SEQ ID NO: 58) (including the unique *Spe* 1 site in the Ad5 genome, bold) and 5' GCG **CTT AAT TAA** CAT CAT CAA TAA TAT ACC TTA TTT T 3' (SEQ ID NO: 59) (including a novel *Pac* 1 site (bold) adjacent to the right Ad5 ITR). This PCR fragment therefore contains nucleotides 27,082 to 35,935 of the Ad5 genome with a deletion of nucleotides 28133 to 32743 (the E3 and fiber genes), and was used to replace the corresponding *Spe* 1/*Pac* 1 fragment of PAdEasy1 to create pDV77.

E. coli strain BJ5183 was electroporated with a mixture of pDV77 and *Pme* 1-linearized pAdTrack as described (He et al., 1998), and DNA was isolated from kanamycin-resistant colonies. The resulting plasmid, pDV83, contains a complete E1-, E3-, and fiber-deleted Ad5 genome with a CMV-driven GFP reporter gene inserted at the site of the E1 deletion. The full-length Ad chromosome was isolated by *Pac* 1 digestion, and transfected to the E1- and fiber-complementing 633 cells (Von Seggern *et al.*, *J. Virol* January 2000). The recovered virus was then plaque purified by plating on 633 cells and stocks were prepared.

Ad5-pseudotyped particles were generated by virus growth in 633 cells, which express the wild type Ad5 fiber protein. Viral particles were isolated and purified over CsCl gradients as previously described (Von Seggern et al., *J. Virol. 73*:1601-1608, 1999). For analysis of viral proteins, ten µg of the purified particles were electrophoresed on 8-16% gradient gels and the protein transferred to nylon membranes. The blot was then probed with rabbit polyclonal antibodies raised against recombinant Ad37 fiber or Ad5 fiber or penton base proteins expressed in baculovirus-infected cells (Figure 27).

Example 11

Construction of a Fiber Expression Construct Containing a Post-Transcriptional Regulatory Element

Previous studies have shown that mRNA transcribed from the woodchuck hepatitis virus (WHV) genome contains an element (the WHV post-transcriptional regulatory element, or WPRE) which can increase expression of a protein encoded by the mRNA via a post-transcriptional mechanism (Loeb *et al., Human Gene Therapy 10*:2295-2305 (1999)). The WPRE has also been shown to enhance expression of transgenes delivered by retroviral vectors. (Zufferey, R. *et al., J. Virol. 73*:2886-2892 (1999)). This example describes the construction of a fiber expression construct (pDV90) containing a WPRE as well as the promoter and TPL sequences as contained in pDV67.

A plasmid (pBS/WPRE) which contains the WPRE was obtained from Dr. Thomas Hope, Salk Institute. Digestion of pBS/WPRE with *Cla*1 releases a 600 bp fragment containing the WPRE (nt 193-1684 of the WHV genome.) Following *Cla*1 digestion, the ends of this fragment were filled by treatment with the large fragment of *E. coli* DNA polymerase 1 in the presence of dNTPs to render them blunt. pDV67 DNA was digested with *Xba*1 (which cuts at a unique site in the transcribed region downstream of the Ad5 fiber open reading frame) and the ends filled by the same treatment. The filled WPRE fragment was then ligated into the filled Xba 1 site of pDV67 to create pDV90 (SEQ ID NO: 65). The sequence is found at GenBank accession no. J04514 (entire genome) in Zufferey, R. *et al., J. Virol. 73*:2886-2892 (1999).

pDV90 was electroporated into E1-2a S8 cells and stable clones expressing fiber isolated as described previously for pDV80.

Example 12

Construction of an Ad5 Fiber Protein with Heterologous Peptide Sequences Inserted in the HI Loop

The receptor-binding knob domain of the Ad5 fiber protein contains several surface loops which are attractive candidates for the insertion of heterologous peptide sequence, as an additional ligand for vector targeting. This example describes the construction of a fiber gene which encodes a fiber protein containing a 6 amino acid peptide linker in the HI loop, and retains the ability to trimerize. The

modified gene also contains a unique novel restriction site at the position of the linker insertion to facilitate addition of the targeting ligand into the HI loop.

The Ad5 fiber gene was amplified from Ad5 genomic DNA (ATCC accession number VR-5) using the primers Fiber ATG (5' TGA AGC GCG CAA GAC CGT CTG AAG 3') (SEQ ID NO: 60) and Fiber TAA (5' CAT AAC **ACT GCA** GAT TCT TTA TTC TTG G 3') (SEQ ID NO: 61), and cloned to the *Nde*1 (filled with the large fragment of *E. coli* DNA polymerase 1 in the presence of dNTPs) and *Pst* 1 sites of pT7-7 using a unique *Pst* 1 site (bold) in the 'Fiber TAA' oligo. The resulting plasmid, pT7/fiber, was digested with *Xba* 1 and *Pst* 1 to excise the fiber gene, which was then cloned into the Pst 1 and Xba 1 sites of pUC119 to create pUC/fiber. This pUC-derived plasmid contains an origin for single-stranded DNA replication and can therefore be used to create template DNA for site-directed mutagenesis.

Site-directed mutagenesis was carried out according to the method of Kunkel (T.A. Kunkel, *PNAS 82*:488-492 (1985)) using the oligonucleotide primer T542 (5' GGT ACA CAG GAA ACA GGA GGT **TCC GGA** GGT GGA GGA GAC ACA ACT CC 3') (SEQ ID NO: 62). This results in the addition of 18 new bases (underlined) encoding the sequence Gly Gly Ser Gly Gly Gly (SEQ ID NO: 63), with a novel BspE1 site (bold) for the addition of further sequences. The inserted sequence is between Thr542 and Gly543 of the Ad5 fiber protein, in the HI loop. The modified plasmid is termed pDV14.

Finally, the modified fiber gene was excised from pDV14 by digestion with Pst 1 and Xba 1 and cloned into the Pst 1 and Xba 1 sites of pGEM3Z (Promega) to create pDV18. *In vitro* transcription/translation experiments with pDV18 (using the TNT™ kit, Promega) demonstrated that the modified fiber gene encoded a protein which was capable of trimerizing.

Alternatively an Ad5 fiber open reading frame (ORF) is amplified from Ad5 genomic DNA (wildtype Ad5 was purchased from the ATCC) using the oligonucleotides 5' ATG **GGA TCC** AAG ATG AAG CGC GCA AGA CCG 3' (SEQ ID NO: 72) and 5' CAT AAC **CTG CAG** GAT TCT TTA TTC TTG GGC 3' (SEQ ID NO:,73) and inserted into the *BamH*I and *Pst* 1 sites of pGEM-3Zf(+) (Promega Inc., Madison, WI) via novel restriction sites (bold type) designed into the primers. The 5' oligonucleotide also contains a G to A change 3 nucleotides 5' of the initial ATG codon (underlined), designed to improve the consensus for translation initiation.

Site-directed mutagenesis is performed by the method of Kunkel (Proc. Nat. Acad. Sci. **82**:488-492 (1985)), using the synthetic oligonucleotide 5' GGT ACA CAG GAA ACA **GGA GGT TCC GGA GGT GGA** GGA GAC ACA ACT CC 3' ((SEQ ID

NO: 74). This operation introduced sequence (bold type) encoding 6 novel amino acids (Gly Gly Ser Gly Gly Gly) immediately following Threonine 542 of the Ad5 fiber, and including a unique restriction site for the insertion of further heterologous sequences (underlined). The resulting plasmid (pDV18A) contains the modified fiber gene under the control of the T7 promoter in the parental pGEM-3Zf(+) and can be used for *in vitro* transcription/translation reactions to produce labeled fiber protein.

Example 13

Use of the Fiber Expression System to Retarget ('Pseudotype') Hybrid Ad/AAV Vectors

Adenoviral vectors which lack essentially all Ad genes ('helper-dependent' or 'gutless' vectors) have recently been developed. In a modification of this idea, vectors ('hybrid' vectors) which contain an adeno-associated virus (AAV) or retroviral genome have been generated. As AAV and retroviral genomes integrate into the chromosome of the target cells, the hybrid Ad/AAV or Ad/retroviral vectors have the potential to provide very long-term gene expression.

Lieber *et al.*, (*J. Virol. 73*(11):9314-9324) describe an Ad vector (Ad.AAV1) which contains an AAV vector genome (a transgene insert flanked by the AAV inverted terminal repeats) inserted into the E1 region. When 293 cells are infected by Ad.AAV1, recombination between the AAV sequences generates a minimal Ad chromosome which carries the Ad inverted terminal repeats and packaging signal flanking the AAV vector genome. This chromosome cannot direct the synthesis of Ad proteins, but can be packaged into Ad vector particles. The remaining unrecombined Ad chromosomes provide the Ad structural proteins in trans, and both the full-length and minimal genomes are packaged into particles. The particles carrying the minimal Ad/AAV hybrid vector are then isolated by CsCl centrifugation.

These particles have the capsid structure of adenovirus, and infect cells using the efficient fiber- and penton base-mediated pathway used by Ad. Following infection, the hybrid genome is able to integrate into the cell's chromosomes by virtue of its AAV sequences. In this example, the AAV vector genome is inserted into the E1 region of a fiber-deleted vector, and the resulting vector is grown in packaging lines expressing either the Ad5 or Ad37 fiber proteins. The particles recovered therefore have the tropisms expected from the respective fiber proteins combined with the ability to integrate their AAV genome into target cells. Such

pseudotyping should be possible with any of a number of modified fiber proteins, as for the fiber-deleted vectors already described by us.

The Ad vector is constructed in a manner analogous to that described for Ad5.βgal.ΔF , by recombination between pAd.AAV1 (Lieber *et al. J. Virol. 73*:9314-9324, 1999) and pDV44 (as described earlier in the specification.) pAd.AAV1 carries an MLV promoter-driven secreted alkaline phosphatase gene (SEAP) as a reporter, and an SV40-driven neomycin phosphotransferase (neo) gene to allow the selection of cells stable transduced by the AAV cassette. The resulting vector (Ad.AAV1.ΦF) has the AAV vector cassette of Ad.AAV1 inserted into the E1 region of a genome with the fiber deletion of Ad5.βgal.ΔF . Growth of Ad.AAV1.ΔF in 633 cells results in particles carrying the AAV genome and the Ad5 fiber, and which have the tropism associated with Ad5. Growth of Ad.AAV1.ΦF in 705 cells produces particles bearing the Ad37 fiber and therefore having its associated different tropism.

Tropism is evaluated by infecting Chang C cells (which express the Ad37 receptor) and A549 cells which do not express this protein but do express the Ad5 receptor (CAR). The extent of infection is monitored by assaying alkaline phosphatase expression, and the fraction of cells stable transduced is assayed by selection with neomycin. By using purified recombinant Ad5 or Ad37 fiber proteins as competitors during infection, the usage of the expected receptors by the pseudotyped particles is evaluated.

Example 14

Use of the Fiber Expression System to Retarget ('Pseudotype') Helper-dependent Ad Vectors

Gutted Ad vectors are those from which most or all viral genes have been deleted. They are grown by co-infection of the producing cells with a "helper" virus (such as using an E1-deleted Ad vector). The helper virus *trans*-complements the missing Ad functions, including production of the viral structural proteins needed for particle assembly. In one embodiment of this invention, the helper virus is a fiber-deleted Ad (such as that described in Von Seggern *et al., J. Virol. 73*:1601-1608 (1999)). The vector is prepared in a fiber expressing cell line such as has been previously described by Von Seggern *et al., J. Gen. Virol. 79*:1461-1468 (1998), Von Seggern *et al., J. Virol. 74*:354-362 (2000). All the necessary Ad proteins except fiber are provided by the fiber-deleted helper virus, and the particles are equipped with the particular fiber expressed by the host cells. A concern with gutted vectors

One way to do this is to mutate the packaging sequence by deleting one or more of the nucleotides comprising the sequence or otherwise mutating the sequence to inactivate or hamper the packaging function. An alternative approach is to engineer the helper genome so that recombinase target sites flank the packaging sequence and to provide a recombinase in the packaging cell. The action of recombinase on such sites results in the removal of the packaging sequence from the helper virus genome. Preferably, the recombinase is provided by a nucleotide sequence in the packaging cell that encodes the recombinase. Most preferably, such sequence is stably integrated into the genome of the packaging cell. Various kinds of recombinase are known by those skilled in the art. The preferred recombinase is Cre recombinase, which operates on so-called lox sites, which are engineered on either side of the packaging sequence as discussed above. Further information about the use of Cre-loxP recombination is found in U.S. Pat. No. 5,919,676 and Morsy and Caskey, *Molecular Medicine Today*, Jan. 1999, pgs. 18-24, both incorporated herein by reference.

This example demonstrates how the fiber-expressing packaging lines can be used to generate pseudotyped particles of helper-dependent or 'gutless' vectors with altered tropisms. As the gutless vectors lack many or all Ad genes, they must be grown as mixed cultures in the presence of a helper virus which can provide the missing functions. To date, such helper viruses have provided all Ad functions except E1, and E1 is complemented by growth in 293 cells or the equivalent. The resulting virus particles are harvested, and the helper virus is typically removed by CsCl gradient centrifugation (the vector chromosome is generally shorter than the helper chromosome, resulting in a difference in buoyant density between the two particles).

An example of a gutless vector is pAdΔRSVDys (Haecker *et al., Human Gene Therapy 7*:1907-1914 (1996)). This plasmid contains a full-length human dystrophin cDNA driven by the RSV promoter and flanked by Ad inverted terminal repeats and packaging signals. 293 cells are infected with a first-generation Ad which serves as a helper virus, and then transfected with purified pAdΔRSVDys DNA. Both the helper Ad genome and the pAdΔRSVDys DNA are replicated as Ad chromosomes, and packaged into particles using the viral proteins produced by the helper virus. Particles are isolated and the pAdΔRSVDys-containing particles separated from the helper by virtue of their smaller genome size and therefore different density on CsCl gradients.

To generate pseudotyped particles containing the pAdΔRSVDys genome, the vector is grown in either 633 or 705 cells and Ad5.βgal.ΔF is used as a helper virus. As in the published method, both the Ad5.βgal.ΔF and AdΔRSVDys genomes replicate and are packaged into particles. The Ad5.βgal.ΔF helper provides all the essential Ad proteins except fiber, and the fiber protein is that produced by the cells (Ad5 fiber in 633 cells and Ad37 fiber in the case of 705 cells). The particles containing AdΔRSVDys genomes are then isolated by centrifugation.

Tropism is evaluated by infecting Chang C cells (which express the Ad37 receptor) and A549 cells which do not express this protein but do express the natural Ad5 receptor (CAR). The extent of infection is assessed by immunofluorescence staining of the infected cells with an anti-dystrophin antibody. By using purified recombinant Ad5 and Ad37 fiber proteins as competitors during infection, the usage of the expected receptors by the pseudotyped particles is evaluated.

Example 15

Targeting EBV-Infected B Cells

There are a number of cell types, such as EBV-transformed B-lymphocytes, that are involved in human disease which are not transducible using standard Ad vectors. To address this problem 'pseudotyped' Ad5.βgal.ΔF particles containing either the wildtype Ad5 fiber protein or a chimeric fiber with the receptor-binding knob domain of the adenovirus type 3 (Ad3) fiber were generated. (Von Seggern *et al., J. Virol.* January, 2000). The strategy used for targeting the B-cells should be broadly applicable for targeting gene delivery to other specific cell types.

Cells and Viruses. THP-1, MRC-5, FaDu, and A-10 cells were purchased from the ATCC. 211B is a 293-derived cell line that expresses the wild-type Ad5 fiber protein (Von Seggern *et al., J. Gen. Virol. 79*:1461-1468 (1998)). E1- 2a (Gorziglia *et al., J. Virol. 70*:4173-4178 (1996)) is an A549-derived cell line which complements adenoviral E1 and E2a functions. The JR, TO, and TL LCL lines were established as described (Huang *et al., Proc. Natl. Acad. Sci. 94*:8156-8161 (1997)

by EBV infection of lymphocytes from three normal donors. THP-1 and all LCL lines were maintained in RPMI 1640 medium (Gibco) + 10% fetal calf serum (FCS) (Hyclone). 211B, MRC-5, and A-10 cells were grown in DMEM + 10% FCS. E1-2a and its derivatives were grown in Richter's modified medium (BioWhitaker) + 10% FCS. Peripheral blood mononuclear cells were isolated from normal human blood (General Clinical Research Center, Scripps Clinic) by sedimentation on Ficoll-Paque (Pharmacia) per the manufacturer's instructions. Wild type Ad2 and Ad3 were purchased from the ATCC. Construction of Ad5.βgal.wt and Ad5.βgal.ΔF (Von Seggern *et al., J. Virol. 73*:1601-1608 (1999)) has been previously described. Av1LacZ4 (Mitttereder *et al., J. Virol. 70*:7498-7509 (1996)) is a first-generation Ad5 vector containing an RSV-driven β-galactosidase reporter gene. Av9LacZ4 (Stevenson *et al., J. Virol. 71*:4782-4790 (1997)) is identical to Av1LacZ4 except that the fiber gene in the vector chromosome was replaced by a recombinant gene encoding a chimeric fiber protein with the receptor-binding domain of the Ad3 fiber (Stevenson *et al., J. virol. 69*:2850-2857 (1995)). Accession numbers for the above are as follows. THP-1: TIB-202, MRC-5: CCL-171, FaDu: HTB-43, A-10: CRL-1476, Ad2: VR-846, Ad3: VR-3.

DNA constructs. The complete Ad5 tripartite leader contained in pDV67 and pDV69 was constructed by assembly of PCR fragments. pDV55 was constructed similar to Example 5. This plasmid contains a 1.2 kb *Bam* HI/*Bgl* II fragment consisting of the first TPL exon, the natural first intron, and the fused second and third TPL exons. Finally, pDV60 was constructed by inserting this TPL cassette into the *Bam* HI site upstream of the Ad5 fiber gene in pcDNA3/Fiber (Von Seggern *et al., J. Gen. Virol. 79*:1461-1468 (1998)). pDV61 and pDV67 were then constructed similar to example 6.

The chimeric Ad3/Ad5 fiber gene was amplified from pGEM5T3H (Stevenson *et al., J.Virol. 69*:2850-2857 (1995) using the primers 5' ATG **GGA TCC** AAG ATG AAG CGC GCA AGA CCG 3'(SEQ ID NO: 75) and 5' CAC TAT **AGC GGC CGC** ATT CTC AGT CAT CTT 3' (SEQ ID NO:76) , and cloned to the *Bam* HI and *Not* I sites of pcDNA3.1/Zeo(+) via novel *Bam* HI and *Not* I sites (bold) engineered into the primers to create pDV68. Finally, the complete TPL fragment described above was then added to the unique *Bam* HI site of this plasmid to create pDV69.

Construction of Stable Cell Lines. E1-2a cells were electroporated as previously described (Von Seggern *et al., J. Gen. Virol. 79*:1461-1468 (1998)) with pDV61, pDV67, or pDV69, and stable lines were selected with 600 μg/ml Zeocin (Invitrogen). Candidate clones were evaluated by immunofluorescence (Von

Seggern *et al., J. Gen. Virol. 79*:1461-1468 (1998)) using a polyclonal antibody generated against the Ad2 fiber (Wickham *et al., Cell 73*:309-319 (1993). Those lines expressing the highest level of nuclear fiber expression were further characterized. Line 601 and 633 were produced by transfection of pDV61 and pDV67, respectively, and therefore express the wildtype Ad5 fiber. Line 644 contains pDV69 and expresses the chimeric 5T3H fiber.

Virus Growth and Analysis. Adenovirus stocks were prepared in the indicated cell lines, and plaque-titered on 633 cells essentially as described (Von Seggern *et al., J. Virol. 73*:1601-1608 (1999)). E1-2a cells (Gorziglia *et al., J. Virol. 70*:4173-4178 (1996). and their derivatives contain a dexamethasone-inducible construct for complementation of E1a. 601, 633, or 644 cells were therefore treated with 0.3 μM dexamethasone for 24 hours prior to infection, and 0.5 μM dexamethasone was included in the overlay for plaque assays. Protein concentration of viral preparations was determined using the BioRad Protein Assay (BioRad) with purified bovine serum albumin as a standard. Particle number was calculated using the formula 1 μg protein = 4×10^9 viral particles. Western blotting was performed as described (Von Seggern *et al., J. Gen. Virol. 79*:1461-1468 (1998)) using polyclonal rabbit antibodies raised against either the Ad2 (Wickham *et al., Cell 73*:309-319 (1993) or Ad3 fibers (Stevenson *et al., J.Virol. 71*:4782-4790 (1997).

Determination of infection and binding to receptor was performed using methods known to those of skill in the art. 2×10^5 cells in a total volume of 200 μl were incubated with the indicated Ad preparation for three hours at 37 °C. Cells were then washed twice with fresh medium, and returned to 37 °C. Two days later, cells were fixed and stained with X-gal and counted by light microscopy as described (Von Seggern *et al., J. Virol. 73*:1601-1608 (1999)). For competition assays, cells were pre-incubated on ice for one hour with either recombinant Ad3 fiber (10 μg/ml) purified from baculovirus or with a crude baculovirus lysate (100 μg/ml) containing the recombinant Ad2 fiber protein (Wickham *et al., Cell 73*:309-319 (1997)). Expression of α_v integrins on cell surfaces was assayed by FACS assay using monoclonal antibodies (the gift of David Cheresh, TSRI) against either $\alpha_v\beta_3$ (LM609) or $\alpha_v\beta_5$ (P1F6) as previously described (Huang *et al., Proc. Natl. Acad. Sci. USA 94*:8156-8161 (1997)). For virus binding assays, CsCl-purified Ad2 or Ad3 was labeled with ^{125}I using Iodogen tubes (Pierce). Free iodine was removed by filtration with a PD-10 Sephadex column (Pharmacia). Cells (1×10^6 cells in a volume of 200 μl either with or without a 100-fold excess of unlabeled virus) were rocked at 4 °C for

two hours with 1 x 10^6 cpm of the labeled virus, washed three times with PBS and counted.

Altered in vitro tropism and infection of B lymphoid cell lines.

Experiments with genetically modified viruses showed that a number of different cell types are more readily infected through interaction with the Ad3 receptor than by the CAR-dependent pathway used by Ad5 (Stevenson *et al., J. Virol. 71*:4782-4790 (1997)). In order to further evaluate the pseudotyping system, the ability of Ad5.βgal.ΔF carrying either the Ad5 or chimeric 5T3H fibers to infect several cell lines was assayed: FaDu (a head and neck tumor line), THP-1 monocytic cells, and MRC-5 fibroblasts were assayed. Consistent with the previous studies (Stevenson et al., J. *Virol. 71*:4782-4790 (1997)), use of the chimeric Ad5/Ad3 fiber protein increased infection of all of these lines at equal particle/cell ratios. In contrast, the rat smooth muscle cell line A-10 was infected somewhat more readily by Ad5- than by Ad3-pseudotyped particles.

Gene delivery to EBV-infected B cells could allow the development of therapies for a variety of lymphoproliferative disorders. For example, *ex vivo* purging of donor marrow to eliminate infected cells could reduce the risk of EBV-associated lymphoproliferative disease, and EBV-induced malignancies such as AIDS-associated lymphoma are also potential targets. However, neither B cells nor EBV-transformed lymphoblastoid cell lines (LCLs) are efficiently infected by Ad5-based vectors. As the tropism of Ad3-pseudotyped particles appeared to be somewhat broader, it was asked whether EBV-infected LCLs could be infected using this system. The ability of Ad3-pseudotyped particles to infect LCLs generated by EBV infection of lymphocytes from three different normal human donors was tested. In agreement with previous reports, there was little or no infection of these by particles carrying the Ad5 fiber. In contrast, virus particles equipped with the chimeric fiber protein were able to efficiently infect all of these lines. At equal particle/cell ratios, all LCLs examined were at least 10-fold more infectible using the Ad3 receptor.

Further studies were performed to correlate the efficiency of infection with the level of attachment and internalization receptors expressed by the cells. The three LCL lines tested all bound very low levels of radiolabeled Ad2 particles, indicating that they expressed little or no CAR. In contrast, all three were able to specifically bind labeled Ad3 particles. This result suggested that fiber receptor distribution was largely responsible for the increased infection of these cells by Ad3-pseudotyped particles. *Selective gene delivery to EBV-infected cells.* The results above

suggested that the minority of EBV-infected B cells present in donor marrow or peripheral blood would be preferentially infected by vectors using the Ad3 receptor. To test this hypothesis, a mixing experiment with normal uninfected peripheral blood mononuclear cells (PBMCs) and EBV-infected cells was performed. JR-LCL cells were mixed at varying ratios with PBMCs isolated from a normal human donor, and the mixture was then infected with Ad5.βgal.ΔF particles containing the 5T3H fiber protein. No infection of normal PBMCs alone was detected. Moreover, the percent of total cells infected increased with the fraction of JR cells added. These experiments indicate that EBV-infected cells can be selectively infected *in vitro* by relatively short (3 hours) exposure to a retargeted Ad vector.

Example 16

Production of Adenovirus Vectors by Addition of Exogenous Fiber

The production of fiberless viruses by growth in a complementing cell line may result in a preparation that also contains contaminating fiber genome resulting from recombination in the complementing cell lines. This disadvantage is eliminated by addition of exogenous fiber to a fiberless adenovirus vector.

Production of fiberless virus by standard methods may include a two-step preparation protocol. This has been described in the earlier examples and is briefly described here again as follows:

Step I - amplification of fiber containing fiberless virus (Ad5/F^-/F^+ or Ad5.βgal.ΔF - fiberless, but there is fiber on the surface, not encoded in genome) on 211B cell line (which stably expresses fiber), followed by CsCl-purification and characterization.

Step II - preparation of virus particles lacking fiber (Ad5F^-) by infection of S.8 cell line with Ad5/F^-/F^+, followed by CsCl purification and characterization. This produces a large stock of particles which do not contain fiber.

Step 1 is necessary because the infection efficiency of fiberless virus is extremely low, e.g. the dose of 20,000 particles/cell of Ad5/βg F^- gives only 10% infected cells.

Contrary to the above, the production of fiberless virus by addition of exogenous fiber involves only a one-step protocol. The fiberless virus is amplified using the S.8 cell line with addition of exogenous fiber into infection media. The

amount of exogenous fiber necessary for production is very low, no more than 75ng of purified fiber required per roller bottle. If desired the process may be followed by CsCl purification. As mentioned above, one advantage to this protocol is that it should provide no chance for recombination of adenovector during preparation.

A 10 roller bottle (RB) preparation of fiberless virus was made using the above two-step procedure. The yield of adenovector was $6.6x10^{12}$ particles - total Ad/βgalF$^-$. A 1 RB preparation of fiberless adenovector was also made from the same initial material using a one-step procedure with exogenous fiber. The total yield was $2.5x10^{11}$ particles - Ad5βgalF$^-$ (one step procedure).

DNA was isolated from both preparations and a PCR assay for fiber contamination was performed. (Figure 28). The PCR assay was developed for detection of very low amounts of fiber contamination, as low as 10^{-18}g. PCR assay showed much lower contamination for the preparation which was done by adding exogenous fiber (10^{-15}g one-step procedure) vs. 10^{-8} two-step procedure). Therefore, less contamination was obtained by simpler one-step approach.

Experiments were done using soluble purified fiber which does not have His-taq on the end (Ad5Fiber = 5F) and with His-taq on the end (Ad5Fiber His = 5FHis). These experiments showed that addition of Ad5Fiber can dramatically increase transduction efficiency of fiberless adenovector by simply adding it exogenously to a fiberless vector. The presence of the His tag on the Ad5FiberHis doesn't have any effect.

The results of these experiments suggest that the fiber is self-assembling with the fiberless vector. This self-assembled virus can then infect cell through the normal entry pathway. (Figure 29) Also, an experiment was done using conditioned media from 633 cell line, which can stably express fiber. A Western blot analysis for 633 condition media, showed that soluble fiber was present in the media during the period of cultivation of this cell line. Presence of soluble fiber in the media gives the possibility to increase transduction efficiency of fiberless adenovector on the HDF cell line. (Figure 30) Because the HDF cell line doesn't have a CAR-receptor, it is especially difficult to transduce this particular cell line, not only with fiberless vector, but also with regular fiber containing adenovector. Different amounts of 633 conditioned media (250µl, 500µl or 1000µl) were added to infectious media during the incubation period with fiberless adenovector.

This experiment also showed a role of soluble fiber in the process of cell entry. The conclusion is that by adding any fiber (wild-type, mutated, with ligand fusions) as long as on has the wild-type shaft (or region necessary to bind penton) one can retarget fiberless vector with any genome inside (gutless, oncolytic, expressing any transgene, etc.) to any cell type that your fiber is specific to. The advantage of this approach is that one does not have to make vectors with each new ligand. Just one fiberless vector need be made that can then be used to make different backbones by adding an exogenous "targetable" fiber off the shelf.

The foregoing specification, including the specific embodiments and examples, is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and modifications can be effected without departing from the true spirit and scope of the present invention. All publications, patents and patent applications cited herein are incorporated by reference in their entirety into the present disclosure.

What is claimed is:

1. An isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third same or different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3.

2. The isolated nucleic acid molecule of claim 1, wherein said sequence is operatively linked to an intron containing an RNA processing signal.

3. The isolated nucleic acid molecule of claim 1 or 2 wherein said TPL nucleotide sequence consists essentially of complete TPL exon 1 operatively linked to complete TPL exon 2 operatively linked to complete TPL exon 3.

4. The isolated nucleic acid molecule of claim 2 wherein said intron is native adenovirus intron 1.

5. The isolated nucleic acid molecule of any one of claims 1 to 4 wherein said TPL nucleotide sequence is shown in SEQ ID NO: 32.

6. The isolated nucleic acid molecule of claim 5 further comprising a promoter and a nucleic acid sequence which encodes an adenoviral structural protein, operatively linked to said promoter and said TPL sequence.

7. The isolated nucleic acid molecule of claim 6 wherein said adenoviral structural protein is a fiber protein or a chimeric protein which includes an adenovirus fiber protein tail domain.

8. The isolated nucleic acid molecule of claim 7 wherein said chimeric protein comprises an Ad3 head domain and an Ad5 tail domain or an Ad5 head domain and an Ad3 tail domain.

9. The isolated nucleic acid molecule of claim 7 wherein said molecule is contained in a plasmid selected from the group consisting of plasmids pCLF, pDV60, pDV67, pDV69, pDV80 and pDV90.

10. The isolated nucleic acid molecule of claim 9 wherein said molecule has a nucleotide sequence selected from the group consisting of sequences shown in SEQ ID NO: 8, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 47, SEQ ID NO: 64 and SEQ ID NO:65.

11. An adenovirus vector complementing plasmid comprising an isolated nucleic acid molecule according to any one of claims 1 to 10.

12. An adenovirus vector packaging cell line comprising a stably integrated nucleic acid molecule as claimed in any one of claims 1 to 11, an operatively-linked promoter and a nucleic acid sequence which encodes an adenovirus structural protein, wherein said TPL sequence consists essentially of a first complete TPL exon operatively linked to a complete second TPL exon operatively linked to a complete third TPL exon.

13. The cell line of claim 12 wherein said first TPL exon is a complete or partial first TPL exon.

14. The cell line of claim 13 wherein said TPL molecule comprises complete TPL exon 1 having the nucleotide sequence of SEQ ID NO: 32 or partial TPL exon 1 having the nucleotide of SEQ ID NO: 26.

15. The cell line of claim 12 wherein said promoter is an inducible promoter.

16. The cell line of claim 12 wherein said adenovirus structural protein is adenovirus fiber protein or a chimeric protein which includes an adenovirus fiber protein tail domain.

17. The cell line of claim 12 wherein said chimeric protein comprises an Ad3 head domain and an Ad5 tail domain or an Ad5 head domain and an Ad3 tail domain.

18. The cell line of claim 12 wherein said nucleic acid molecule is selected from the group consisting of plasmids pDV60, pDV67, pDV69, pDV80 and pDV90.

19. The cell line of claim 18 wherein said nucleic acid molecule has a nucleotide sequence from the group consisting of sequences shown in SEQ ID NO: 43, SEQ ID NO: 44 and SEQ ID NO: 47.

20. The cell line of claim 12 wherein said cell line is an epithelial cell line.

21. The cell line of claim 20 wherein said cell line supports the production of a recombinant adenovirus vector genome by complementation of a deficient viral gene in said vector genome.

22. The cell line of claim 21 wherein said cell line further produces an adenovirus protein and thereby complements a deficient adenovirus gene in said vector genome, and wherein said cell line complements an adenovirus early protein gene and a fiber gene.

23. The cell line of claim 22 wherein the deletion of said deficient adenovirus gene is complemented by the expression of said gene under the control of an inducible promoter.

24. A recombinant adenovirus particle comprising a recombinant adenovirus vector genome wherein said genome does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene in a packaging cell, wherein said packaging cell comprises an isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide sequence, and optionally an exogenous protein, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third same or different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3.

25. The recombinant adenovirus particle of claim 24 wherein said adenovirus vector genome does not encode one or more functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4 protein.

26. The particle of claim 24 wherein said adenovirus vector genome is Ad5.Bgal.ΔF.

27. The particle of claim 24 wherein said adenovirus vector genome is contained in the adenovirus particle deposited under ATCC accession # VR2636 and corresponding to Ad5.Bgal.ΔF.

28. The particle of claim 24 wherein said particle lacks fiber protein or contains a modified fiber protein.

29. The particle of claim 24 wherein said particle comprises an adenovirus fiber protein or a chimeric protein having an adenovirus fiber protein tail domain, said chimeric protein comprising an Ad3 head domain and an Ad5 tail domain or an Ad5 head domain and an Ad3 tail domain.

30. The particle of claim 24 wherein said exogenous protein is a therapeutic gene product.

31. A helper-independent fiberless recombinant adenovirus vector genome comprising genes which:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle in a packaging cell without complementation of said fiber gene or said genome lacks at least the fibre gene, wherein said packaging cell comprises an isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide sequence, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third same or different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3. and

(b) encode an exogenous protein.

32. The adenovirus vector genome of claim 31 wherein said adenovirus vector genome does not encode one or more functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4 protein.

33. The adenovirus vector genome of claim 31 wherein said adenovirus vector genome is Ad5.Bgal. ΔF.

34. The adenovirus vector genome of claim 33 wherein said adenovirus vector genome has a nucleotide sequence shown in SEQ ID NO:27 and corresponds to Ad5.Bgal. ΔF.

35. The adenovirus vector genome of claim 31 wherein said adenovirus vector genome is contained in the adenovirus particle deposited under ATCC accession VR-2636 corresponding to Ad5.Bgal. ΔF.

36. The adenovirus vector genome of claim 31 wherein said exogenous protein is a therapeutic gene product.

37. An isolated nucleic acid that comprises the adenovirus vector genome of claim 31.

38. A method for producing an adenovirus vector particle containing a helper-independent fiberless recombinant adenovirus vector genome, said method comprising providing a packaging cell line which complements replication and packaging of said genome and a helper-independent fiberless recombinant adenovirus vector genome which is deficient in expressing sufficient functional fiber protein to support assembly of fiber-containing particles, wherein said packaging cell comprises an isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and, wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3, and harvesting said particles produced by said cell line.

39. The method of claim 38 wherein said packaging cell line complements adenovirus fiber protein.

40. The method of claim 38 wherein said adenovirus vector genome comprises genes that:

(a) express all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

(b) express an exogenous protein.

41. The method of claim 38 wherein said packaging cell line comprises a stably integrated first nucleic acid molecule alternatively operatively linked to a promoter, and said first nucleic acid is operatively linked to a second nucleic acid molecule encoding an adenovirus structural protein, wherein said first nucleic acid molecule comprises an adenovirus tripartite leader (TPL) nucleotide sequence operatively linked to an intron containing an RNA processing signal, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and wherein said isolated nucleic acid molecule may not comprise the nucleic acid sequence consisting of partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3..

42. The method of claim 38 wherein said helper-independent fiberless recombinant adenovirus vector genome is introduced by infecting said cell line with a virus particle containing said genome.

43. The method of claim 42 wherein said particle is a particle comprising a helper-independent recombinant adenovirus vector genome comprising genes that:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

(b) encode an exogenous protein,

wherein said particle comprises an adenovirus fiber protein or a chimeric protein that includes an adenovirus fiber protein tail domain.

44. The method of claim 38 wherein said helper-independent fiberless recombinant adenovirus vector genome is introduced into said cell line by transfecting said cell line with said helper-independent fiberless recombinant adenovirus vector genome

45. The method of claim 44 wherein said adenovirus vector genome comprises genes which:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

(b) encode an exogenous protein.

46. The method of claim 38 wherein said packaging cell line is transfected with a nucleic acid molecule encoding adenovirus fiber protein.

47. The method of claim 46 wherein said nucleic acid molecule is a nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide sequence, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and said molecule further comprises a sequence encoding adenovirus fiber protein.

48. The method of claim 39 wherein said adenovirus fiber protein is a modified fiber protein.

49. The method of claim 38 further comprising the step of coating said particle with adenovirus fiber protein.

50. A method for delivery of an exogenous gene to a target cell comprising contacting said cell with an amount of a recombinant adenovirus particle of claim 24 sufficient to infect said cell.

51. The method of claim 50 wherein said exogenous gene encodes a therapeutic gene product.

52. The method of claim 51 wherein said recombinant adenovirus particle contains a modified fiber protein which binds a preselected target cell and directs delivery of the particle to said target cell.

53. The method of claim 50 wherein said recombinant adenovirus particle comprises a helper-independent fiberless recombinant adenovirus vector genome comprising genes that:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

(b) encode an exogenous protein.

54. The method of claim 50 wherein said modified fiber protein has an amino terminal head domain which binds to α_v integrins and thereby targets cells with α_v integrin receptors.

55. The method of claim 50 wherein said contacting is conducted *in vitro.*

56. The method of claim 55 wherein said contacting is conducted on cells of a tissue which are first removed from the body of a patient, and the cells are subsequently returned to said patient.

57. The method of claim 50 wherein said contacting is conducted *in vivo* by administering said recombinant adenovirus particle to a tissue of said patient.

58. The method of claim 57 wherein said administering is intravenously, intraperitoneally, by aerosol, topically or by injection.

59. A method for pseudotyping recombinant viral vectors comprising complementing a missing fiber gene of a helper-independent or helper dependent fiberless recombinant adenovirus vector genome by expressing in packaging cells a

fiber gene from a different adenoviral serotype than said recombinant adenovirus vector, thereby pseudotyping said vector.

60. A method for specifically targeting an adenovirus vector to a cell of choice comprising introducing a helper-independent or helper-dependent fiberless recombinant adenovirus vector genome into a packaging cell line for producing a fiber gene-deleted adenovirus vector, wherein said gene for a missing fiber protein is complemented with a gene for a desired modification for targeting the vector to a cell of choice.

61. A method for producing a modified adenovirus comprising providing *in vitro* an exogenous fiber protein to a fiberless adenovirus.

62. The method of claim 61, wherein said fiber is provided by adding fiber protein in a suitable buffer to a fiberless virus preparation, thereby producing a modified adenovirus.

63. The method of claim 61, wherein a helper-independent or helper-dependent fiberless recombinant adenovirus vector genome is introduced into a packaging cell line to produce a fiberless adenovirus to which exogenous fiber protein will be provided.

64. A method for delivering a heterologous gene to EBV-infected B cells comprising infecting said B cells with a pseudotyped Ad5βgal.ΔF particle or other fiber-deleted adenovirus particle, said particle having a chimeric fiber including the receptor-binding knob domain of the adenovirus type 3 fiber.

65. The adenovirus particle of claim 24 wherein said adenovirus vector genome lacks a fibre gene or lacks a portion of the fibre gene sequence such that fibre protein is not expressed in sufficient quantities to support packaging.

66. The recombinant adenovirus particle of claim 24 comprising a helper-independent recombinant adenovirus vector genome comprising genes that:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the gene encoding fibre, and

(b) optionally encodes an exogenous protein.

67. The recombinant adenovirus particle of claim 24 wherein said particle further comprises a nucleic acid encoding an exogenous protein.

68. A method for producing a modified adenovirus comprising providing a packaging cell line for producing a fiberless adenovirus helper-dependent fiberless recombinant adenovirus vector genome and a helper virus vector, wherein said cell line complements at least a deficient fiber protein gene, thereby producing the modified adenovirus.

69. The packaging cell line of claim 9 wherein said cell line is selected from the group consisting of 293, A549, W163, HeLa, Vero, 211, 211A and an epithelial cell line comprising the stably integrated nucleic acid molecule.

70. The recombinant adenovirus particle of claim 21 wherein said exogenous protein is selected from a group consisting of a tumor-suppressor protein, a biologically active fragment thereof, a suicide protein and a biologically active fragment thereof.

71. A composition for preparing a therapeutic vector, said composition comprising a plasmid comprising an adenovirus genome lacking a nucleotide sequence encoding a fiber protein or a genome that is incapable of expressing sufficient fiber to result in packaging.

72. A method of delivering a heterologous gene to a human or any animal comprising providing said heterologous gene to a target cell wherein said target cell is contacted *in vivo* or *ex vivo* with an amount of a recombinant adenovirus particle of claim 24 sufficient to infect said cell and thereby deliver the heterologous gene.

73. The recombinant adenovirus vector particle of either claim 24 or 31, wherein no fiber protein is expressed.

74. The recombinant adenovirus vector particle of claim 24, wherein said genome expresses insufficient fiber to allow incorporation of said protein into the particle such that the particle cannot use the fiber pathway for infection.

75. The recombinant adenovirus genome of claim 31, wherein said genome expresses insufficient fiber to allow incorporation of said protein into a particle such that the particle cannot use the fiber pathway for infection.

76. A method for producing a gutless adenoviral vector particle comprising:

a) delivering a helper adenovirus vector genome to an adenovirus vector packaging cell, wherein said helper adenovirus vector genome lacks any gene encoding adenovirus fiber protein or lacks the ability to encode sufficient adenovirus fiber protein to produce an adenoviral vector comprising fiber protein in the absence of complemetation by said packing cell and wherein said packaging cell comprises the nucleic acid molecule of claim 2 operably linked to a promoter and to an adenoviral fiber protein or to a chimeric protein that includes an adenovirus fiber protein tail domain;

(b) delivering a gutless adenovirus vector genome to said packaging cell; and

(c) recovering the gutless adenoviral vector particle produced by said cell.

77. The method of claim 76, wherein said helper adenovirus vector genome is delivered by viral infection.

78. The method of claim 77, wherein said gutless adenovirus vector genome is delivered by transfection.

79. The method of claim 76, wherein said gutless adenovirus vector genome comprises an operable packaging sequence.

80. The method of claim 79, wherein said helper adenovirus vector genome has a mutation in its packaging sequence that renders said genome substantially incapable of being packaged as an adenoviral vector particle by said packaging cell.

81. The method of claim 79, wherein said helper adenovirus vector genome comprises recombinase sites flanking its packaging sequence and said packaging cell further comprises a nucleotide sequence encoding a recombinase.

82. The method of claim 81, wherein said recombinase site is a lox site and said recombinase is Cre.

83. A helper adenovirus particle comprising an adenovirus vector genome that does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene, wherein said genome has a mutation in its packaging sequence that renders said genome substantially incapable of being packaged.

84. The helper adenovirus particle of claim 83, wherein said mutation comprises a deletion of at least one nucleotide in said packaging sequence.

85. The helper adenovirus particle of claim 84, wherein said adenovirus vector genome does not encode functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3, and E4 proteins.

86. A helper adenovirus particle comprising an adenovirus vector genome with recombinase sites flanking its packaging sequence, wherein said vector genome does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene.

87. The helper adenovirus particle of claim 86, wherein said adenovirus vector genome does not encode functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3, and E4 proteins.

88. An adenovirus particle comprising a gutless adenoviral vector genome and a fiberless capsid.

89. An adenovirus particle comprising a gutless adenoviral vector genome and a capsid comprising a modified fiber protein.

90. A packaging cell for the production of a fiberless or fiber-modified gutless adenovirus particle comprising an adenovirus vector complementing plasmid and a nucleotide sequence encoding a recombinase, wherein said complementing plasmid

comprises the nucleic acid molecule of claim 2 operably linked to a promoter and to a nucleotide sequence encoding an adenoviral fiber protein or a chimeric adenoviral fiber protein.

91. The packaging cell of claim 90, wherein said complementing plasmid and said nucleotide sequence encoding a recombinase are stably integrated into the genome of said cell.

92. The packaging cell of claim 90, further comprising a helper adenovirus vector genome.

93. The packaging cell of claim 90, wherein said recombinase is Cre..

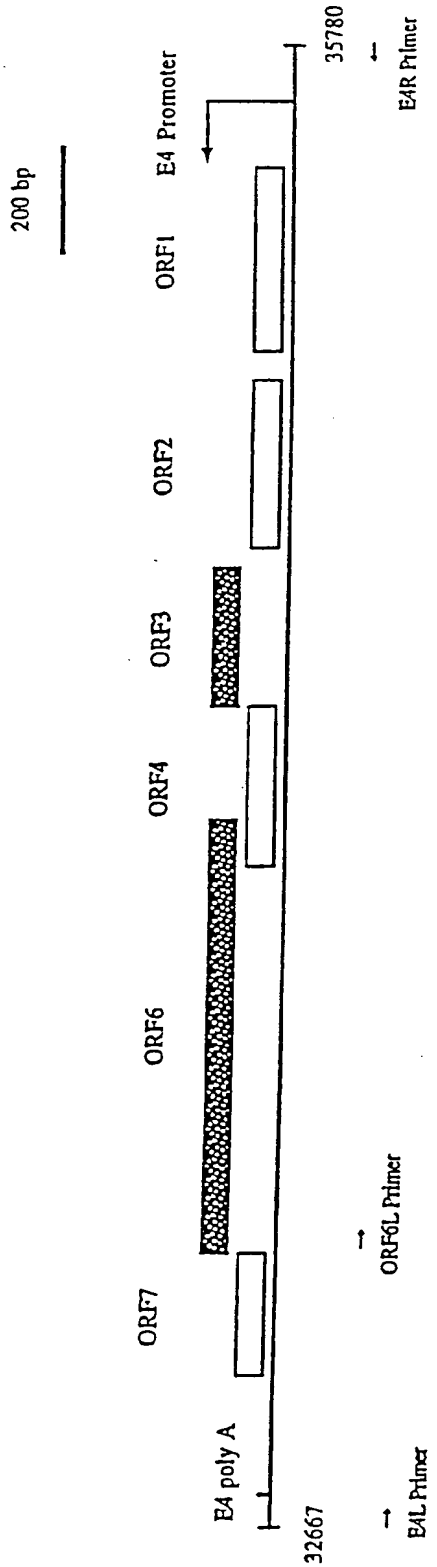
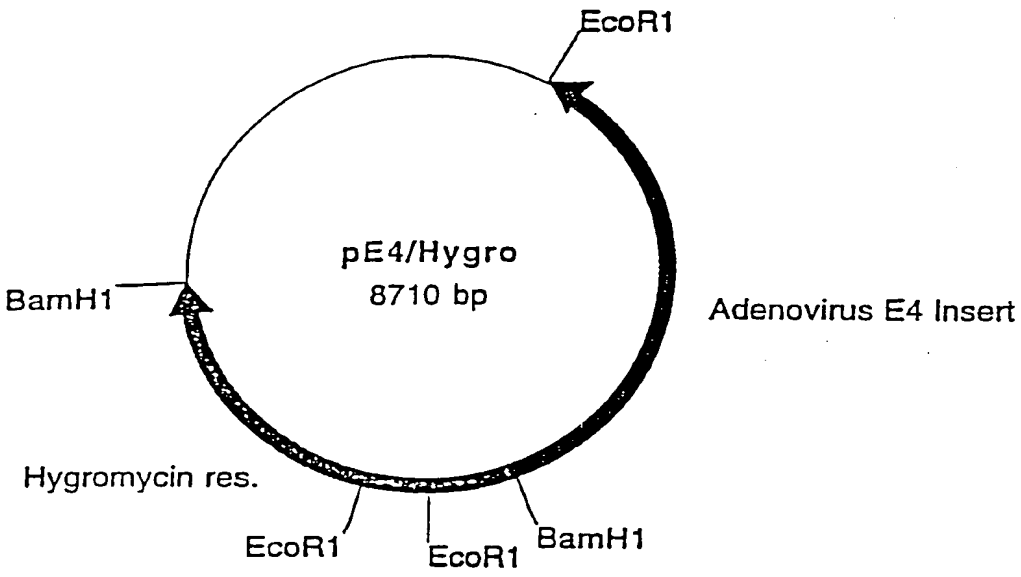

200 bp
E4 Promoter
35780
E4R Primer
ORF1
ORF2
ORF3
ORF4
ORF6
ORF7
ORF6L Primer
E4 poly A
32667
E4L Primer

FIG. 1


EcoR1
pE4/Hygro
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BamH1
Adenovirus E4 Insert
Hygromycin res.
EcoR1
EcoR1
BamH1

FIG. 2

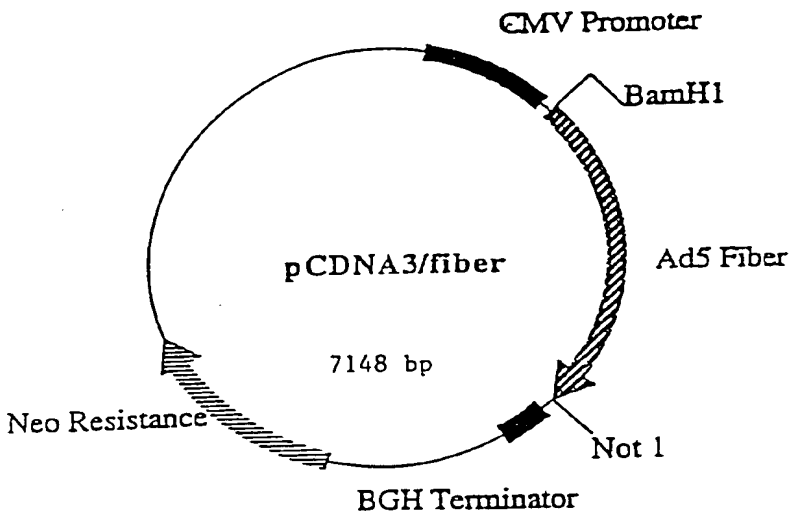

CMV Promoter
BamH1
pCDNA3/fiber
7148 bp
Ad5 Fiber
Neo Resistance
Not 1
BGH Terminator

FIG. 3

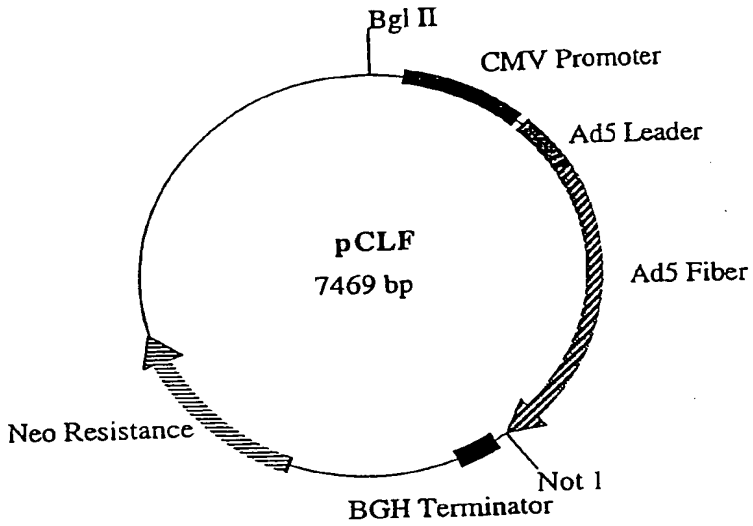

Bgl II
CMV Promoter
Ad5 Leader
pCLF
7469 bp
Ad5 Fiber
Neo Resistance
Not 1
BGH Terminator

FIG. 4

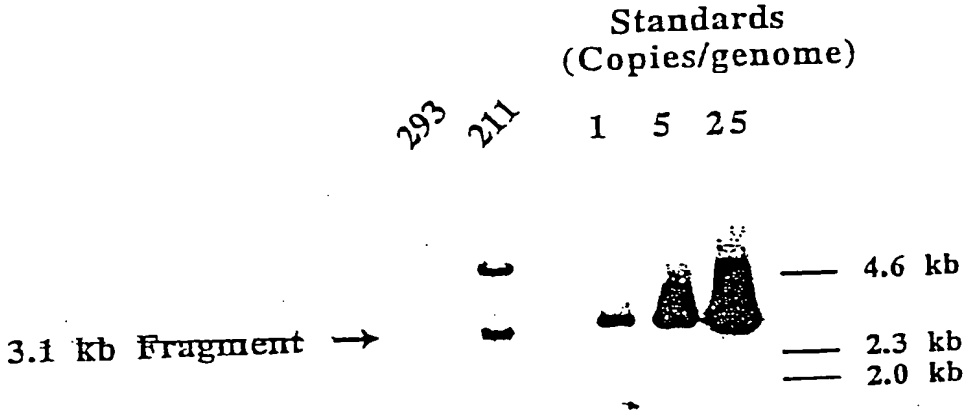

Standards
(Copies/genome)
293
211
1
5
25
4.6 kb
3.1 kb Fragment
2.3 kb
2.0 kb

FIG. 5

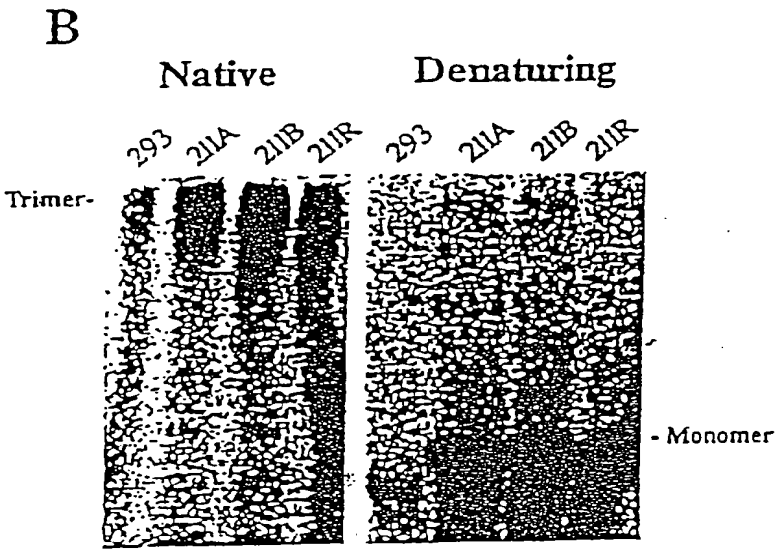

B
Native
Denaturing
293
211A
211B
211R
293
211A
211B
211R
Trimer-
- Monomer

FIG. 6

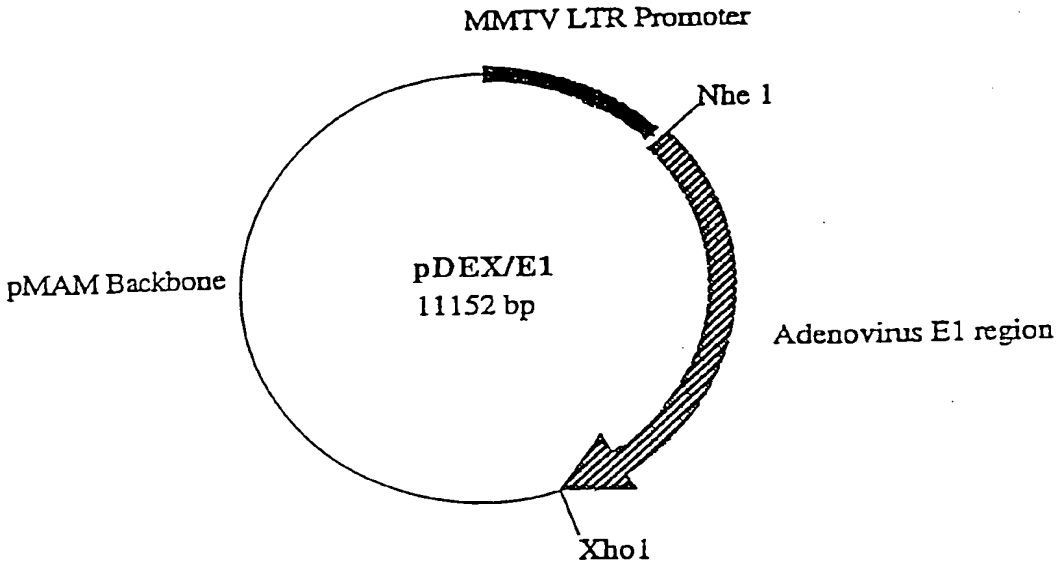

MMTV LTR Promoter
Nhe 1
pMAM Backbone
pDEX/E1
11152 bp
Adenovirus E1 region
Xho1

FIG. 7

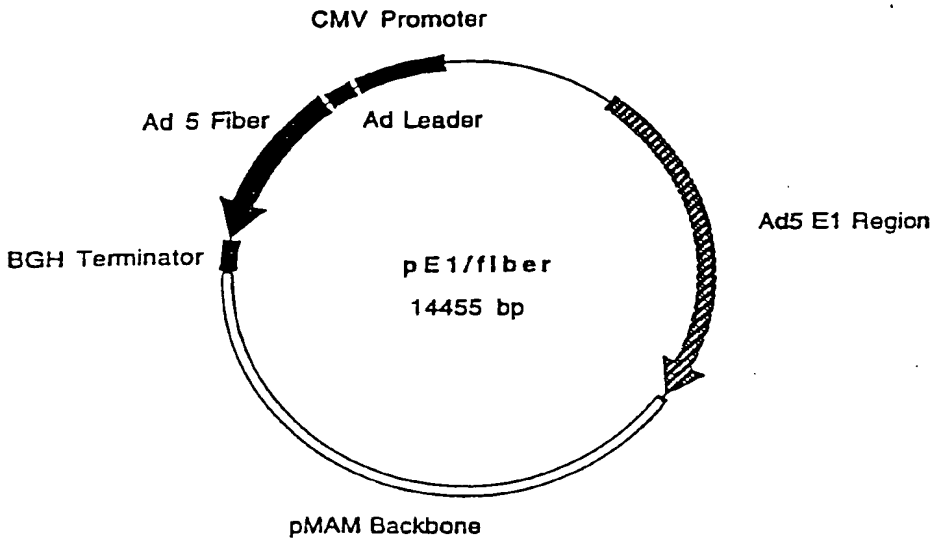

CMV Promoter
Ad 5 Fiber
Ad Leader
Ad5 E1 Region
BGH Terminator
pE1/fiber
14455 bp
pMAM Backbone

FIG. 8

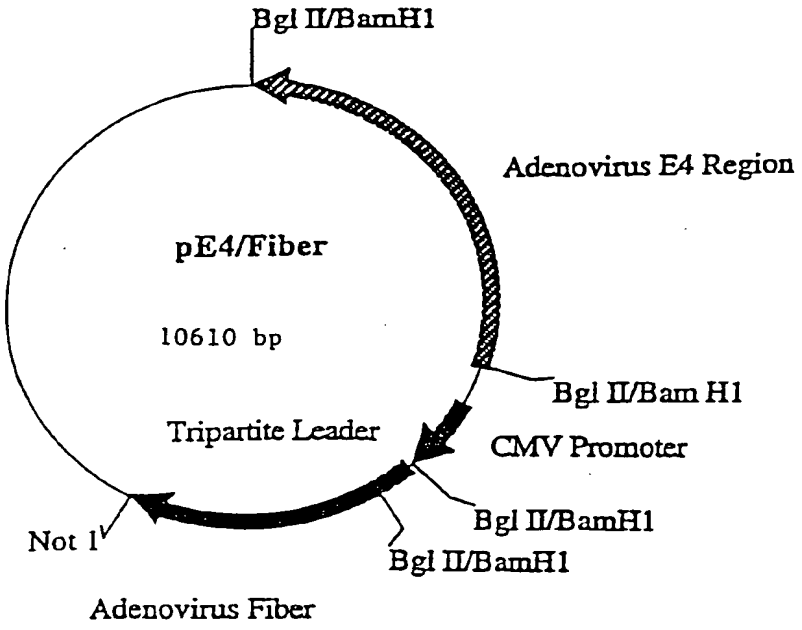

Bgl II/BamH1
Adenovirus E4 Region
pE4/Fiber
10610 bp
Bgl II/Bam H1
Tripartite Leader
CMV Promoter
Bgl II/BamH1
Not 1
Bgl II/BamH1
Adenovirus Fiber

FIG. 9

pΔE1Bβgal

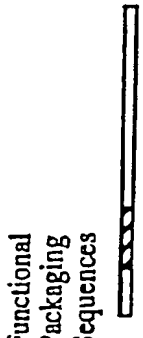


FIG. 10

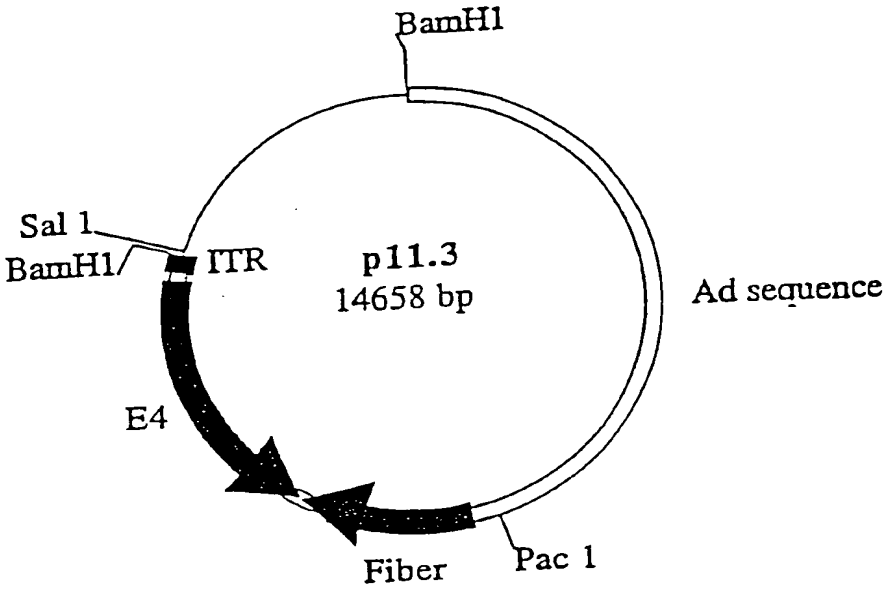
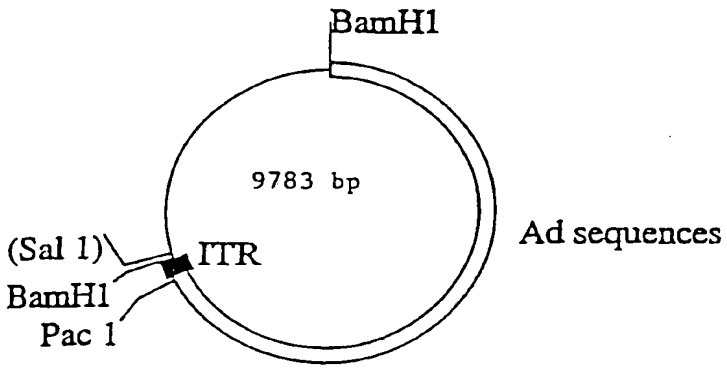


FIG. 11



p8.2

FIG. 12

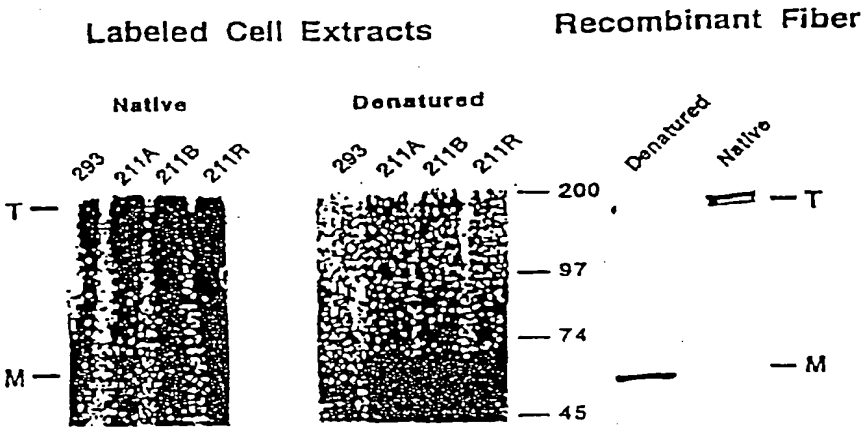

Labeled Cell Extracts
Recombinant Fiber
Native
Denatured
293
211A
211B
211R
293
211A
211B
211R
Denatured
Native
T
M
200
97
74
45
T
M

FIG. 13

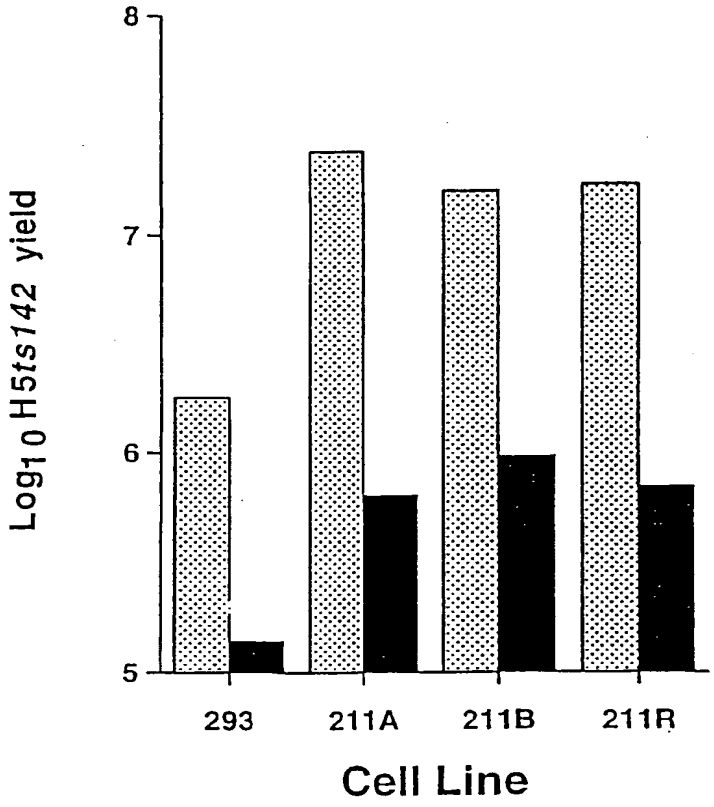


FIG. 14

A


Ad2/5
Ad3
Ad19/37
Ad40 (1)
Ad40 (2)

B

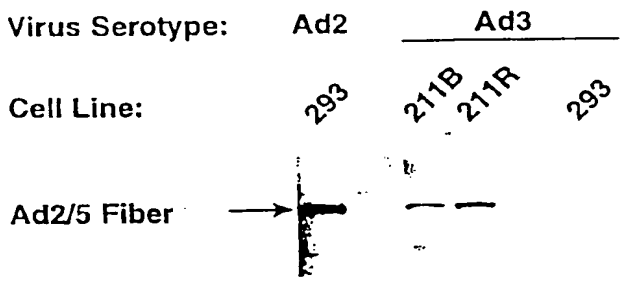

Virus Serotype:
Ad2
Ad3
Cell Line:
293
211B
211R
293
Ad2/5 Fiber

FIG. 15

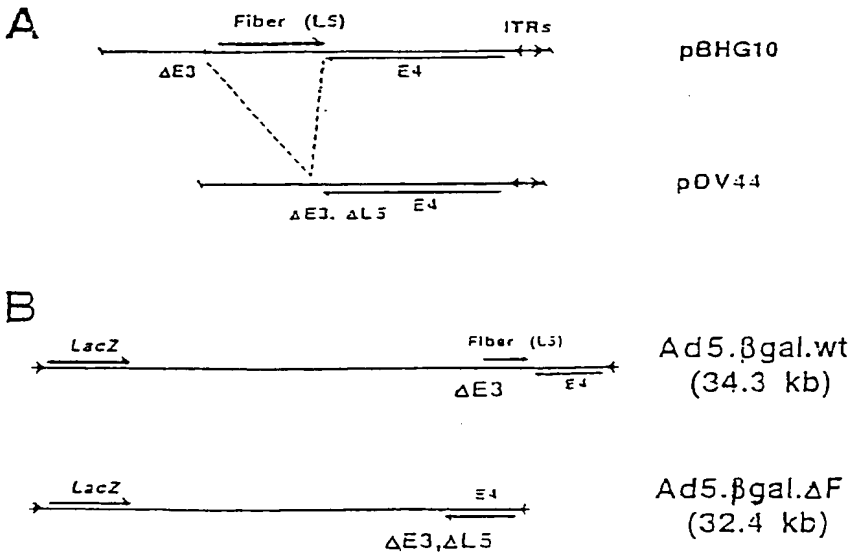

A
Fiber (L5)
ITRs
ΔE3
E4
pBHG10
pDV44
ΔE3, ΔL5
E4
B
LacZ
Fiber (L5)
ΔE3
E4
Ad5.βgal.wt
(34.3 kb)
LacZ
E4
ΔE3,ΔL5
Ad5.βgal.ΔF
(32.4 kb)

FIG. 16

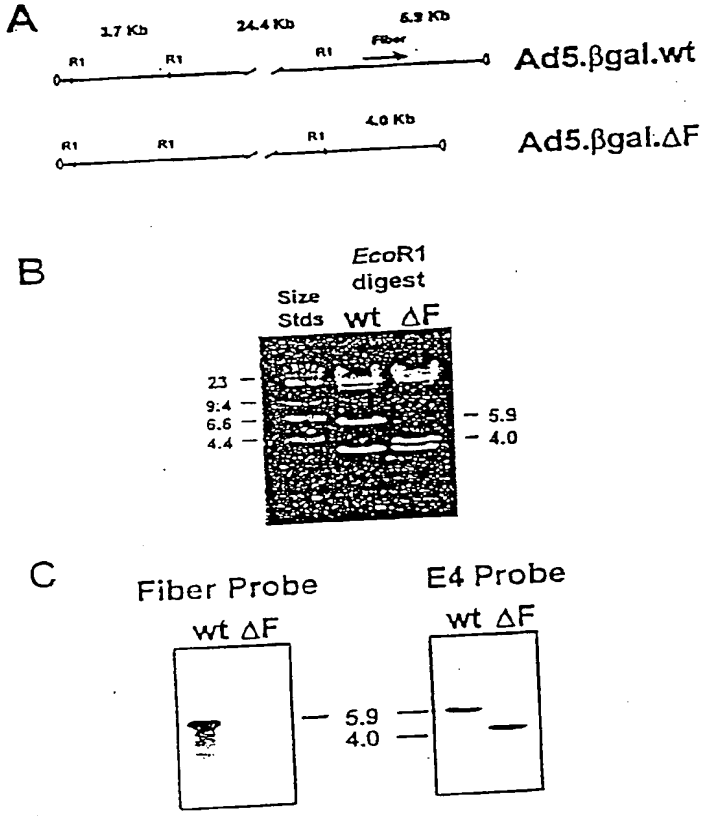

A
1.7 Kb
24.4 Kb
5.9 Kb
Fiber
R1
R1
R1
Ad5.βgal.wt
4.0 Kb
R1
R1
R1
Ad5.βgal.ΔF
B
EcoR1
digest
Size
Stds
wt
ΔF
23
9.4
6.6
4.4
5.9
4.0
C
Fiber Probe
wt ΔF
E4 Probe
wt ΔF
5.9
4.0

FIG. 17

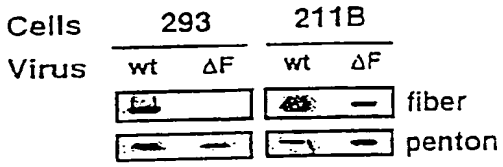
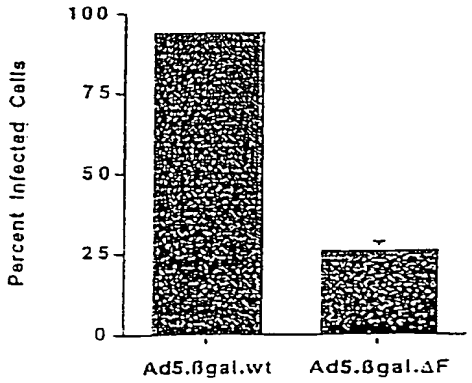


FIG. 18

A


100
75
50
25
0
Percent Infected Cells
Ad5.ßgal.wt
Ad5.ßgal.ΔF

B

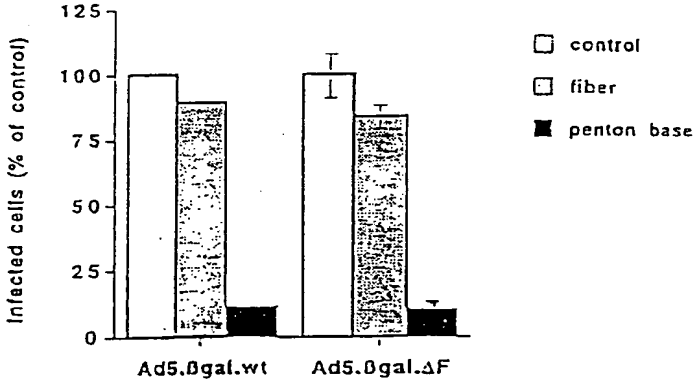

125
100
75
50
25
0
Infected cells (% of control)
Ad5.ßgal.wt
Ad5.ßgal.ΔF
control
fiber
penton base

FIG. 19

Improved Fiber-complementing Cell Lines

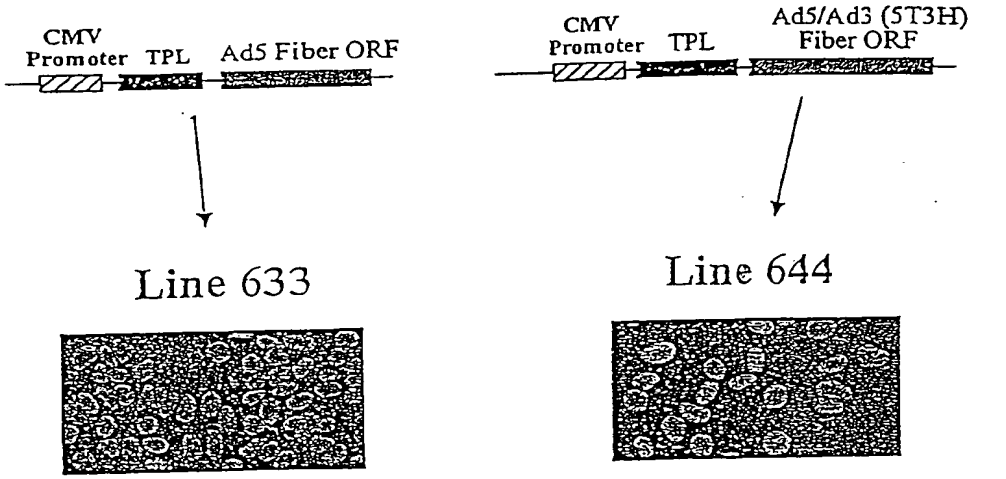


FIG. 20

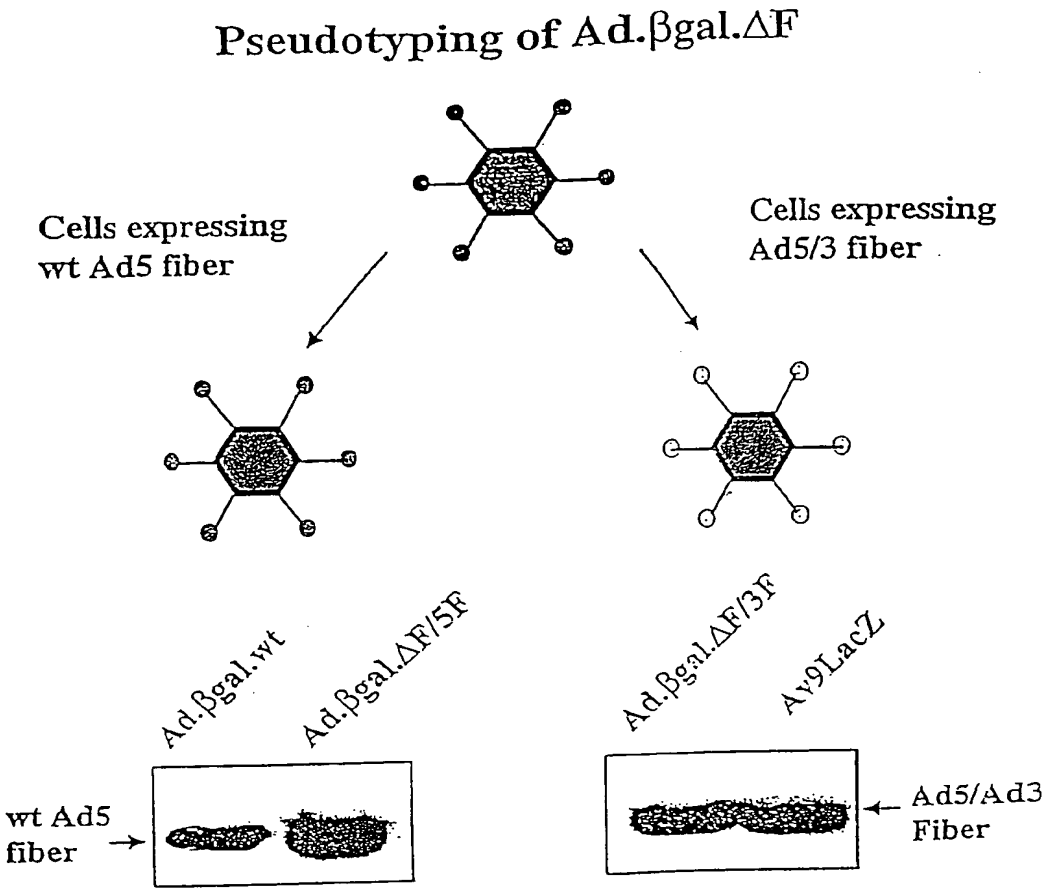

Pseudotyping of Ad.βgal.ΔF
Cells expressing wt Ad5 fiber
Cells expressing Ad5/3 fiber
Ad.βgal.wt
Ad.βgal.ΔF/5F
Ad.βgal.ΔF/3F
Av9LacZ
wt Ad5 fiber
Ad5/Ad3 Fiber

FIG. 21

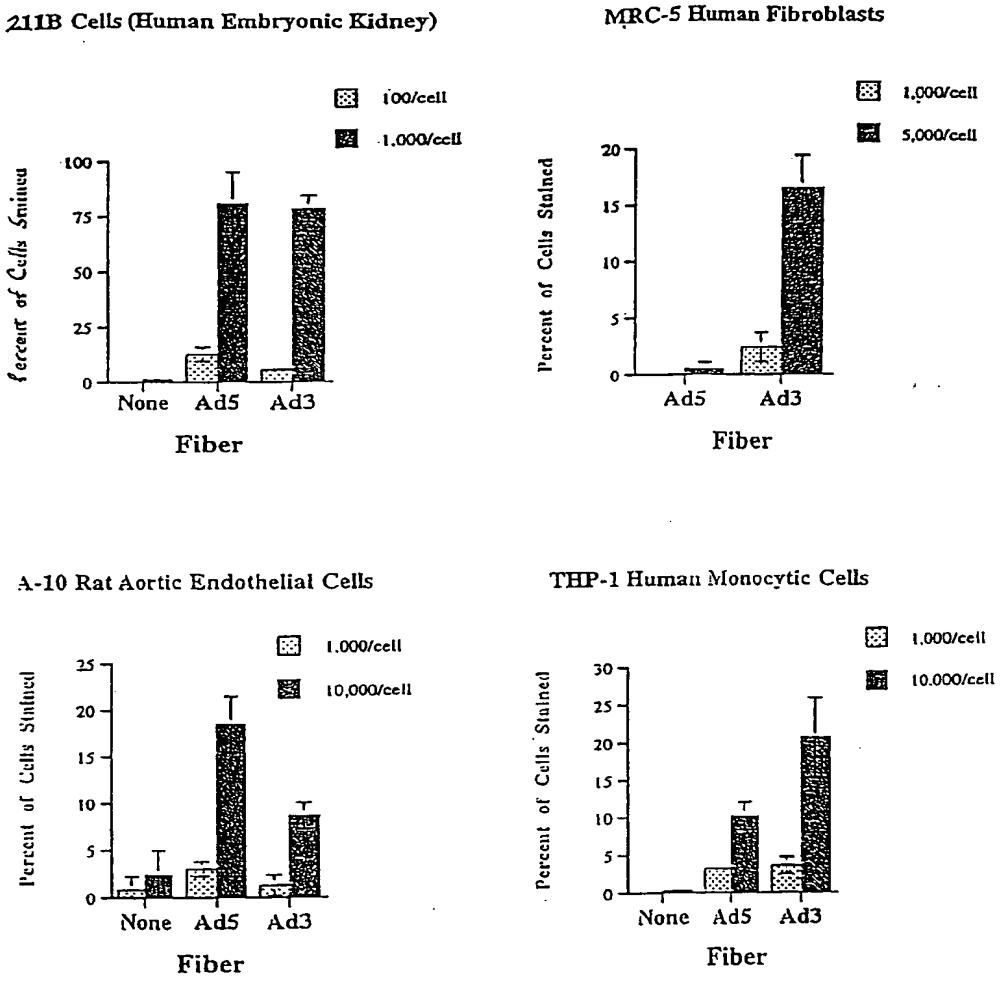

211B Cells (Human Embryonic Kidney)
100/cell
1,000/cell
Percent of Cells Stained
100
75
50
25
0
None
Ad5
Ad3
Fiber
MRC-5 Human Fibroblasts
1,000/cell
5,000/cell
Percent of Cells Stained
20
15
10
5
0
Ad5
Ad3
Fiber
A-10 Rat Aortic Endothelial Cells
1,000/cell
10,000/cell
Percent of Cells Stained
25
20
15
10
5
0
None
Ad5
Ad3
Fiber
THP-1 Human Monocytic Cells
1,000/cell
10,000/cell
Percent of Cells Stained
30
25
20
15
10
5
0
None
Ad5
Ad3
Fiber

FIG. 22

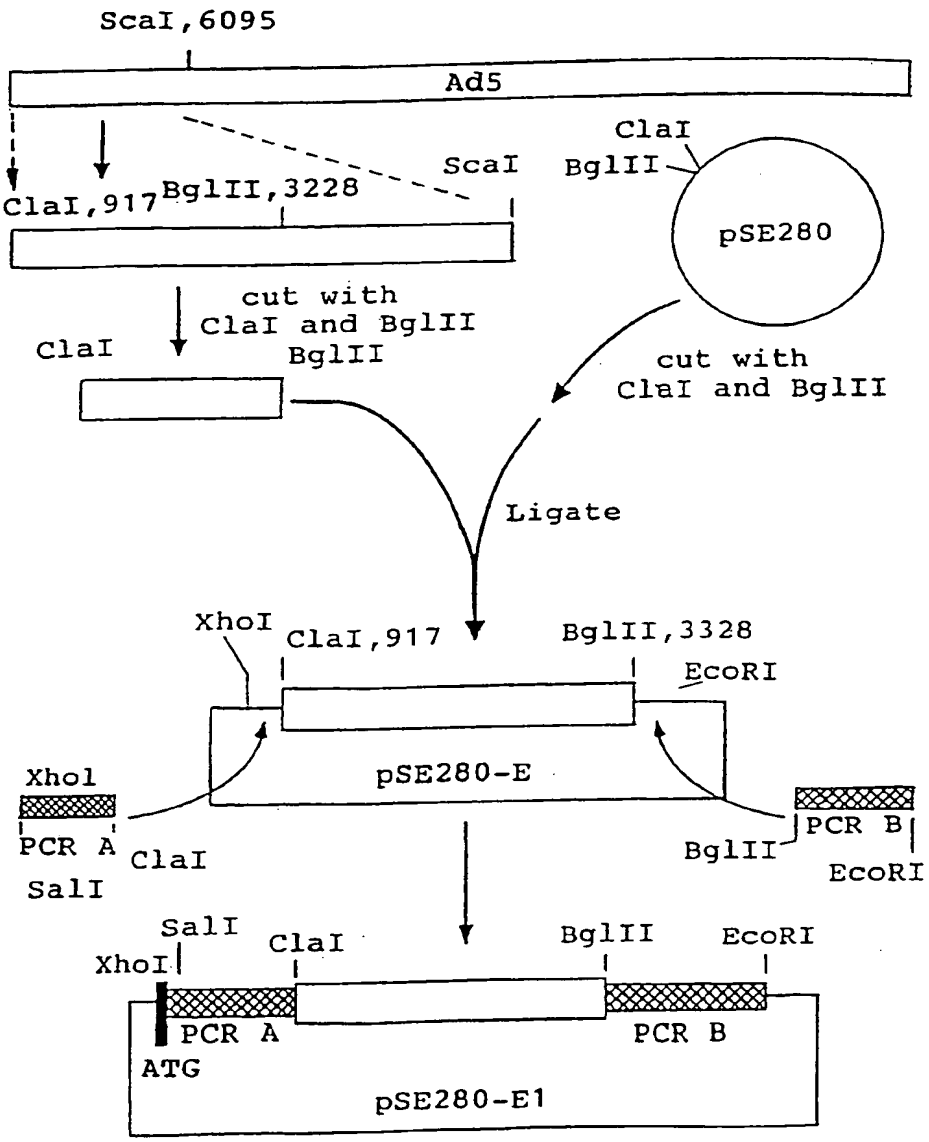

ScaI,6095
Ad5
ClaI,917
BglII,3228
ScaI
ClaI
BglII
pSE280
cut with
ClaI and BglII
ClaI
BglII
cut with
ClaI and BglII
Ligate
XhoI
ClaI,917
BglII,3328
EcoRI
pSE280-E
XhoI
PCR A
ClaI
SalI
BglII
PCR B
EcoRI
SalI
ClaI
BglII
EcoRI
XhoI
PCR A
PCR B
ATG
pSE280-E1

FIG. 23

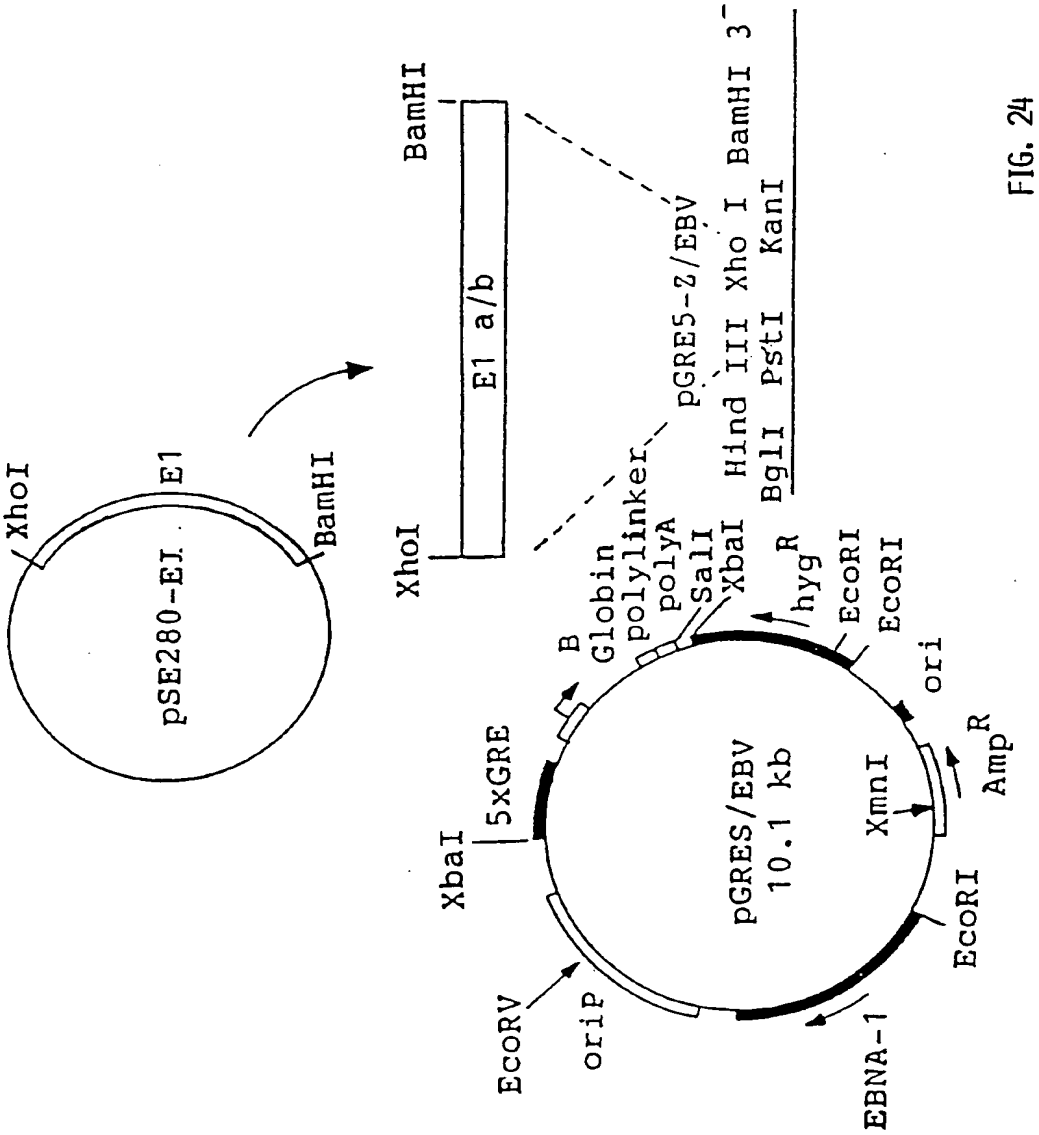

XhoI
E1
BamHI
pSE280-E1
BamHI
E1 a/b
XhoI
pGRE5-Z/EBV
Hind III Xho I BamHI 3'
BglI PstI KanI
B Globin polylinker
polyA
SalI
XbaI
hygR
EcoRI
EcoRI
ori
AmpR
XmnI
EcoRI
EBNA-1
pGRES/EBV
10.1 kb
XbaI
5xGRE
EcoRV
oriP

FIG. 24

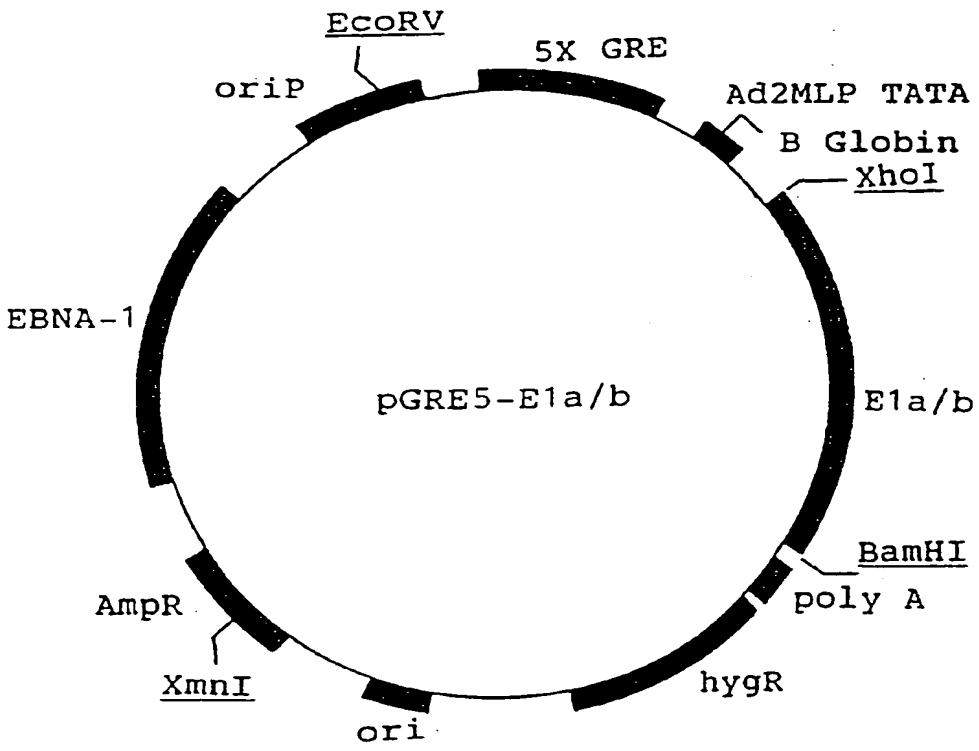

EcoRV
5X GRE
oriP
Ad2MLP TATA
B Globin
XhoI
EBNA-1
pGRE5-E1a/b
E1a/b
BamHI
poly A
AmpR
XmnI
hygR
ori

FIG. 25

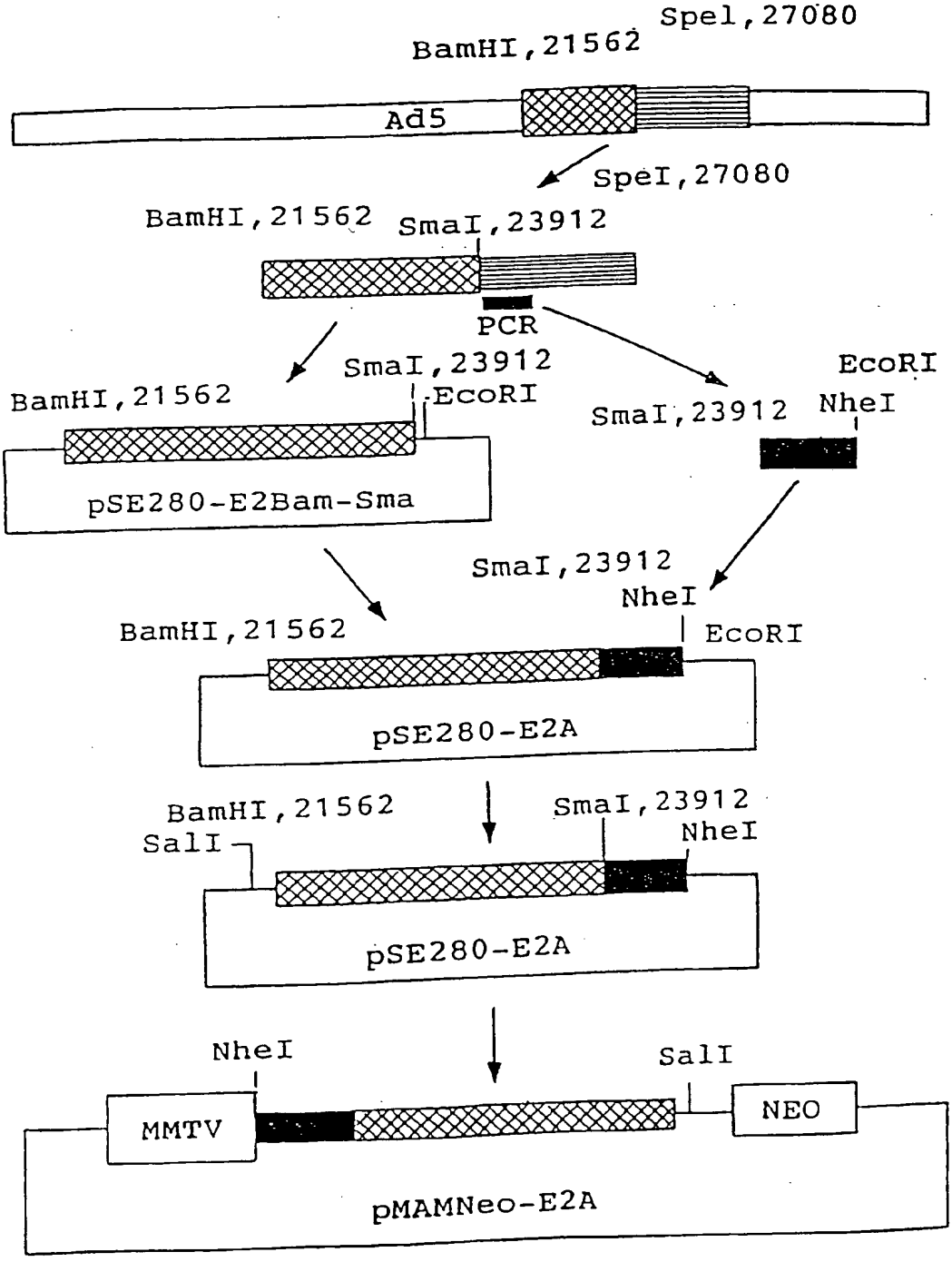

SpeI,27080
BamHI,21562
Ad5
SpeI,27080
BamHI,21562
SmaI,23912
PCR
SmaI,23912
EcoRI
BamHI,21562
EcoRI
SmaI,23912
NheI
pSE280-E2Bam-Sma
SmaI,23912
NheI
BamHI,21562
EcoRI
pSE280-E2A
BamHI,21562
SmaI,23912
SalI
NheI
pSE280-E2A
NheI
SalI
MMTV
NEO
pMAMNeo-E2A

FIG. 26

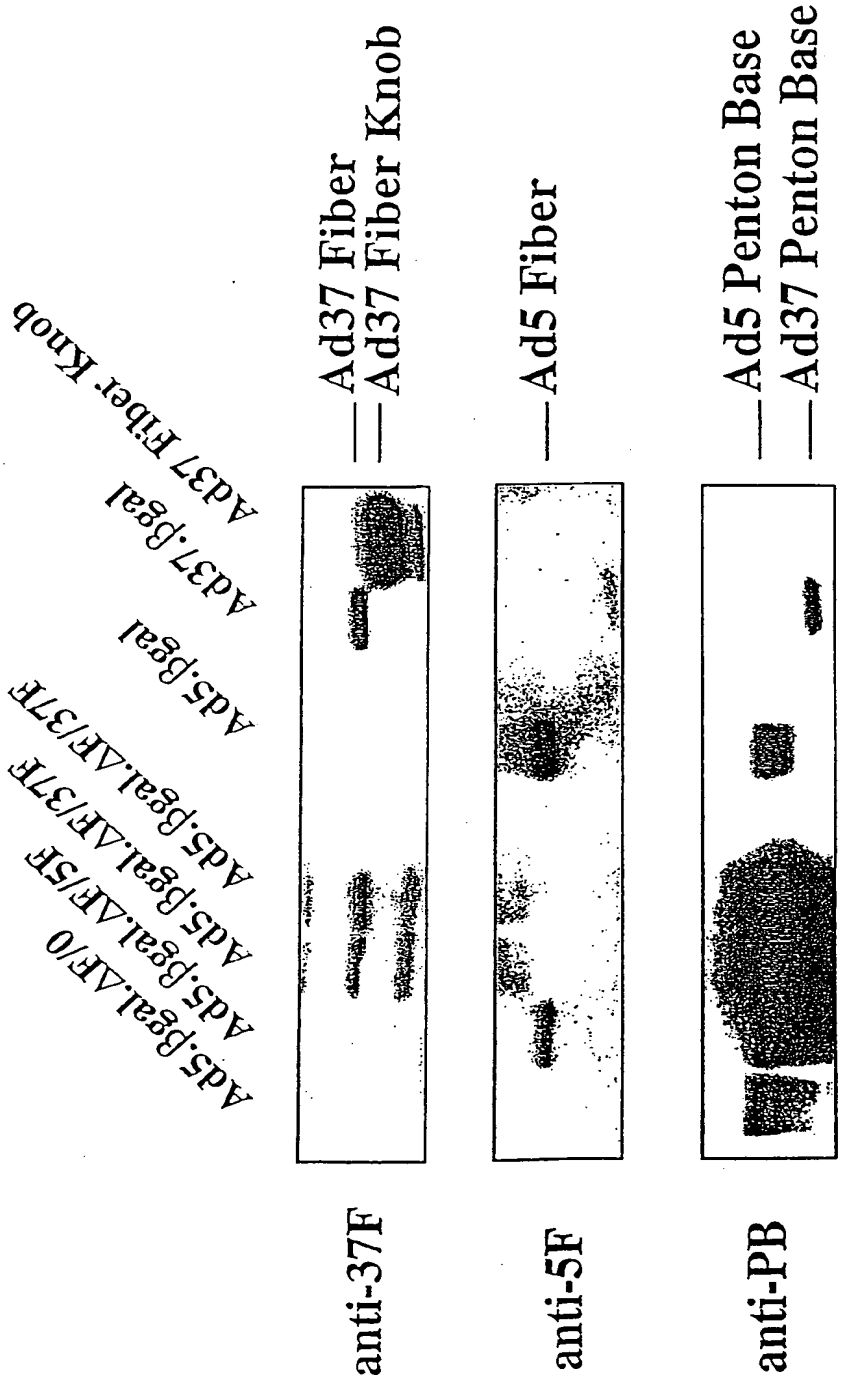
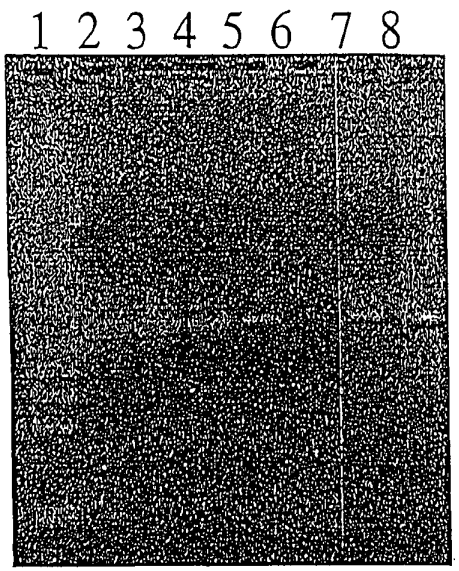

—Ad37 Fiber
—Ad37 Fiber Knob
—Ad5 Fiber
—Ad5 Penton Base
—Ad37 Penton Base
anti-37F
anti-5F
anti-PB
Ad5.βgal.ΔF/0
Ad5.βgal.ΔF/5F
Ad5.βgal.ΔF/37F
Ad5.βgal.ΔF/37F
Ad5.βgal
Ad37.βgal
Ad37 Fiber Knob

FIG. 27

PCR analysis for fiber presence contamination of fiberless adenonovectors.



1. 100 bp ladder

2. positive control - 10ng

3.positive control - 1ng

4positive control - 1pg

5. Positive control - 1fg

6. Positive control -10^{-18}g

7. Ad5BgF- one step preparation with soluble fiber on s.8 cell line

8.Ad5BgF- two step preparation (211B and S.8 cell lines).

FIG. 28

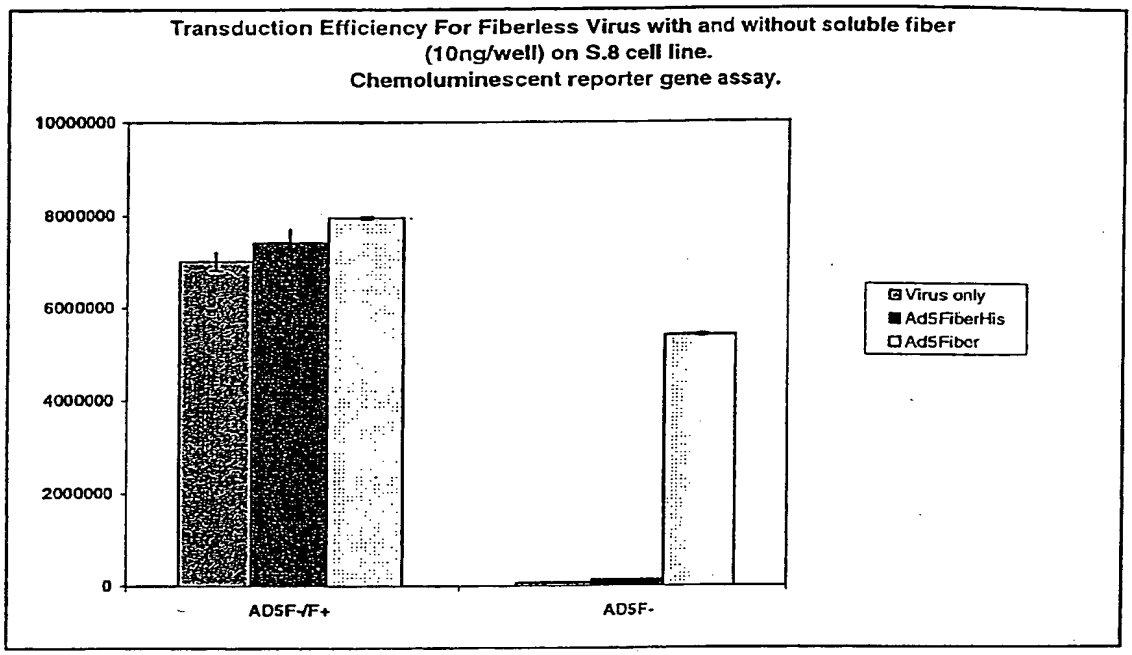

Transduction Efficiency For Fiberless Virus with and without soluble fiber
(10ng/well) on S.8 cell line.
Chemoluminescent reporter gene assay.
10000000
8000000
6000000
4000000
2000000
0
AD5F-/F+
AD5F-
Virus only
Ad5FiberHis
Ad5Fiber

FIG. 29

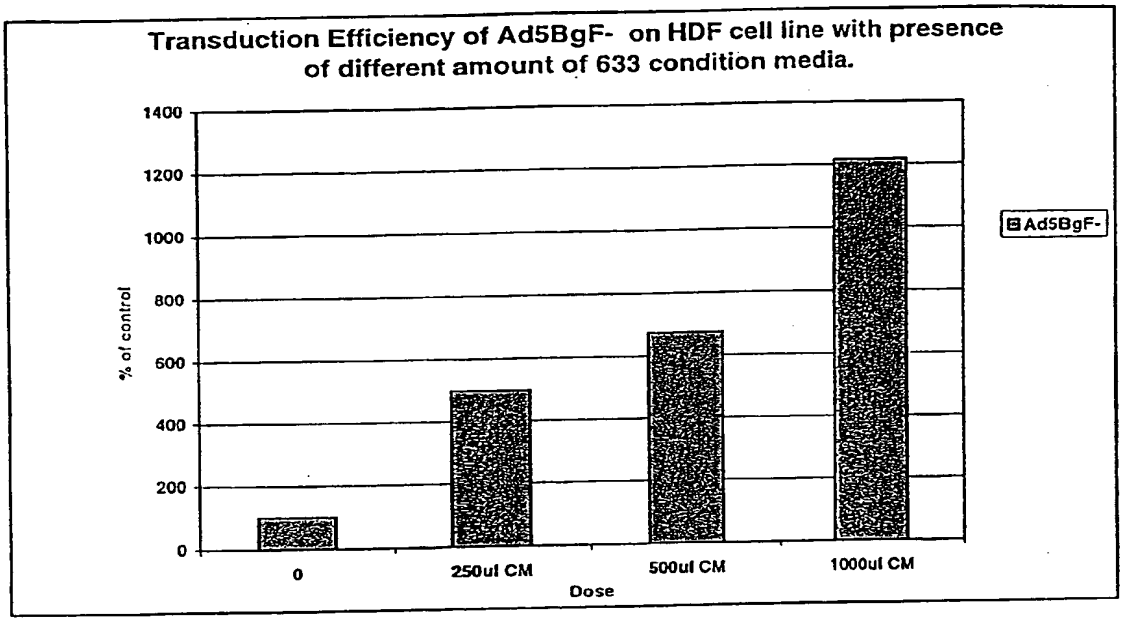

Transduction Efficiency of Ad5BgF- on HDF cell line with presence of different amount of 633 condition media.
1400
1200
1000
800
600
400
200
0
% of control
0
250ul CM
500ul CM
1000ul CM
Dose
Ad5BgF-

FIG. 30

SEQUENCE LISTING

<110> Novartis Ag
The SCRIPPS RESEARCH INSTITUTE

<120> ADENOVIRUS VECTORS, PACKAGING CELL LINES, COMPOSITIONS, AND METHODS FOR PREPARATION AND USE

<130> 1294.0010001

<140>
<141>

<160> 76

<170> PatentIn Ver. 2.1

<210> 1
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 1
cggtacacag aattcaggag acacaactcc 30

<210> 2
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 2
gcctggatcc gggaagttac gtaacgtggg aaaac 35

<210> 3
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: linker

<400> 3
cgcggatccg cg 12

<210> 4
<211> 8710
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: plasmid

<400> 4
cacctaaatt gtaagcgtta atattttgtt aaaattcgcg ttaaattttt gttaaatcag 60
ctcatttttt aaccaatagg ccgaaatcgg caaaatccct tataaatcaa aagaatagac 120
cgagataggg ttgagtgttg ttccagtttg gaacaagagt ccactattaa agaacgtgga 180
ctccaacgtc aaagggcgaa aaaccgtcta tcagggcgat ggcccactac gtgaaccatc 240
accctaatca agtttttttgg ggtcgaggtg ccgtaaagca ctaaatcgga accctaaagg 300
gagcccccga tttagagctt gacggggaaa gccggcgaac gtggcgagaa aggaagggaa 360
gaaagcgaaa ggagcgggcg ctagggcgct ggcaagtgta gcggtcacgc tgcgcgtaac 420
caccacaccc gccgcgctta atgcgccgct acagggcgcg tcccattcgc cattcaggct 480
gcgcaactgt tgggaagggc gatcggtgcg ggcctcttcg ctattacgcc agctggcgaa 540
aggggatgt gctgcaaggc gattaagttg ggtaacgcca gggttttccc agtcacgacg 600
ttgtaaaacg acggccagtg aattgtaata cgactcacta tagggcgaat tgggtaccgg 660
gccccccctc gaggtcgacg gtatcgataa gcttgatatc gaattcagga gacacaactc 720
caagtgcata ctctatgtca ttttcatggg actggtctgg ccacaactac attaatgaaa 780
tatttgccac atcctcttac acttttttcat acattgccca agaataaaga atcgtttgtg 840
ttatgtttca acgtgtttat ttttcaattg cagaaaattt caagtcattt ttcattcagt 900
agtatagccc caccaccaca tagcttatac agatcaccgt accttaatca aactcacaga 960
accctagtat tcaacctgcc acctccctcc caacacacag agtacacagt cctttctccc 1020
cggctggcct taaaaagcat catatcatgg gtaacagaca tattcttagg tgttatattc 1080
cacacggttt cctgtcgagc caaacgctca tcagtgatat taataaactc cccgggcagc 1140
tcacttaagt tcatgtcgct gtccagctgc tgagccacag gctgctgtcc aacttgcggt 1200
tgcttaacgg gcggcgaagg agaagtccac gcctacatgg gggtagagtc ataatcgtgc 1260
atcaggatag ggcggtggtg ctgcagcagc gcgcgaataa actgctgccg ccgccgctcc 1320
gtcctgcagg aatacaacat ggcagtggtc tcctcagcga tgattcgcac cgcccgcagc 1380
ataaggcgcc ttgtcctccg ggcacagcag cgcaccctga tctcacttaa atcagcacag 1440
taactgcagc acagcaccac aatattgttc aaaatcccac agtgcaaggc gctgtatcca 1500
aagctcatgg cggggaccac agaacccacg tggccatcat accacaagcg caggtagatt 1560
aagtggcgac ccctcataaa cacgctggac ataaacatta cctcttttgg catgttgtaa 1620
ttcaccacct cccggtacca tataaacctc tgattaaaca tggcgccatc caccaccatc 1680
ctaaaccagc tggccaaaac ctgcccgccg gctatacact gcagggaacc gggactggaa 1740
caatgacagt ggagagccca ggactcgtaa ccatggatca tcatgctcgt catgatatca 1800
atgttggcac aacacaggca cacgtgcata cacttcctca ggattacaag ctcctcccgc 1860
gttagaacca tatcccaggg aacaacccat tcctgaatca gcgtaaatcc cacactgcag 1920
ggaagacctc gcacgtaact cacgttgtgc attgtcaaag tgttacattc gggcagcagc 1980
ggatgatcct ccagtatggt agcgcgggtt tctgtctcaa aaggaggtag acgatcccta 2040
ctgtacggag tgcgccgaga caaccgagat cgtgttggtc gtagtgtcat gccaaatgga 2100
acgccggacg tagtcatatt tcctgaagca aaaccaggtg cgggcgtgac aaacagatct 2160
gcgtctccgg tctcgccgct tagatcgctc tgtgtagtag ttgtagtata tccactctct 2220
caaagcatcc aggcgccccc tggcttcggg ttctatgtaa actccttcat gcgccgctgc 2280
cctgataaca tccaccaccg cagaataagc cacacccagc caacctacac attcgttctg 2340
cgagtcacac acgggaggag cgggaagagc tggaagaacc atgttttttt ttttattcca 2400
aaagattatc caaaacctca aaatgaagat ctattaagtg aacgcgctcc cctccggtgg 2460
cgtggtcaaa ctctacagcc aaagaacaga taatggcatt tgtaagatgt tgcacaatgg 2520
cttccaaaag gcaaacggcc ctcacgtcca agtggacgta aaggctaaac ccttcagggt 2580
gaatctcctc tataaacatt ccagcacctt caaccatgcc caaataattc tcatctcgcc 2640
accttctcaa tatatctcta agcaaatccc gaatattaag tccggccatt gtaaaaatct 2700
gctccagagc gccctccacc ttcagcctca agcagcgaat catgattgca aaaattcagg 2760
ttcctcacag acctgtataa gattcaaaag cggaacatta acaaaaatac cgcgatcccg 2820
taggtccctt cgcagggcca gctgaacata atcgtgcagg tctgcacgga ccagcgcggc 2880
cacttccccg ccaggaacct tgacaaaaga acccacactg attatgacac gcatactcgg 2940
agctatgcta accagcgtag ccccgatgta agctttgttg catgggcggc gatataaaat 3000
gcaaggtgct gctcaaaaaa tcaggcaaag cctcgcgcaa aaaagaaagc acatcgtagt 3060
catgctcatg cagataaagg caggtaagct ccggaaccac cacagaaaaa gacaccattt 3120
ttctctcaaa catgtctgcg ggtttctgca taaacacaaa ataaaataac aaaaaaacat 3180
ttaaacatta gaagcctgtc ttacaacagg aaaaacaacc cttataagca taagacggac 3240
tacggccatg ccggcgtgac cgtaaaaaaa ctggtcaccg tgattaaaaa gcaccaccga 3300
cagctcctcg gtcatgtccg gagtcataat gtaagactcg gtaaacacat caggttgatt 3360
catcggtcag tgctaaaaag cgaccgaaat agcccggggg aatacatacc cgcaggcgta 3420
gagacaacat tacagccccc ataggaggta taacaaaatt aataggagag aaaaacacat 3480

aaacacctga aaaaccctcc tgcctaggca aaatagcacc ctcccgctcc agaacaacat 3540
acagcgcttc acagcggcag cctaacagtc agccttacca gtaaaaaaga aaacctatta 3600
aaaaaacacc actcgacacg gcaccagctc aatcagtcac agtgtaaaaa agggccaagt 3660
gcagagcgag tatatatagg actaaaaaat gacgtaacgg ttaaagtcca caaaaaacac 3720
ccagaaaacc gcacgcgaac ctacgcccag aaacgaaagc caaaaaaccc acaacttcct 3780
caaatcgtca cttccgtttt cccacgttac gtaacttccc ggatccgcgg cattcacagt 3840
tctccgcaag aattgattgg ctccaattct tggagtggtg aatccgttag cgaggtgccg 3900
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

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<211> 30
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

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<210> 7
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<212> DNA

<213> Artificial Sequence

<220>
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<220>
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<210> 29
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
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<210> 30
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<212> DNA
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<220>
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<220>
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<223> Description of Artificial Sequence: primer

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<223> Description of Artificial Sequence: primer

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<220>
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<223> Description of Artificial Sequence: plasmid

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<211> 8238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: plasmid

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<400> 51
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<220>
<223> Description of Artificial Sequence: primer

<400> 52
ctgacaaact cagatcttgt ttattg 26

<210> 53
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 53
gtcgactcta gaggatccag a 21

<210> 54
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 54
ccggactcta gatggcaacc atggcgctac 30

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<220>
<223> Description of Artificial Sequence: primer

<400> 55
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<210> 56
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

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<220>
<223> Description of Artificial Sequence: primer

<400> 57
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<220>
<223> Description of Artificial Sequence: primer

<400> 58
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<210> 59
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<220>
<223> Description of Artificial Sequence: primer

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<210> 60
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<220>
<223> Description of Artificial Sequence: primer

<400> 60
tgaagcgcgc aagaccgtct gaag 24

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<220>
<223> Description of Artificial Sequence: primer

<400> 61
cataacactg cagattcttt attcttgg 28

<210> 62
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<220>
<223> Description of Artificial Sequence: primer

<400> 62
ggtacacagg aaacaggagg ttccggaggt ggaggagaca caactcc 47

<210> 63
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic peptide

<400> 63
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<210> 64
<211> 7231
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: plasmid

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<223> Description of Artificial Sequence: oligonucleotide

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<223> Description of Artificial Sequence: primer

<400> 76
cactatagcg gccgcattct cagtcatctt 30

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/00265

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C07K14/075 C12N5/10 C12N15/34 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 13499 A (CIBA GEIGY AG ;SCRIPPS RESEARCH INST (US); MEMEROW GLEN R (US); VO) 2 April 1998 (1998-04-02) the whole document	1-6, 9-14, 21-24, 38,39, 46,47, 49-52,59
X	WO 97 37220 A (CHUGAI BIOPHARMACEUTICALS INC) 9 October 1997 (1997-10-09) page 44-49	10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 May 2000	06/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/00265

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17783 A (UNIV MICHIGAN) 30 April 1998 (1998-04-30) page 16, line 14 -page 21, line 14; examples 1-8	1-5,11, 24,30, 36,72, 76-78, 90-93
A	WO 95 00655 A (UNIV MCMASTER) 5 January 1995 (1995-01-05) cited in the application examples 1-3	24-26
X	STEVENSON S C ET AL: "Selective targeting of human cells by a chimeric adenovirus vector containing a modified fiber protein" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 71, no. 6, June 1997 (1997-06), pages 4782-4790, XP002121965 ISSN: 0022-538X the whole document	7,8
X	VON SEGGERN DAN J ET AL., : "Complementation of a fibre mutant adenovirus by packaging cell lines stably expressing the adenovirus type 5 fibre protein" JOURNAL OF GENERAL VIROLOGY, vol. 79, June 1998 (1998-06), page 1461-1468 XP002138278 cited in the application the whole document	1-4,6, 11-13, 15, 20-24, 31, 38-53, 59-63, 65-75
X	WO 96 39530 A (UNIV PENNSYLVANIA ;WILSON JAMES M (US); FISHER KRISHNA J (US); GAO) 12 December 1996 (1996-12-12) page 3, line 21 -page 5, line 9 examples 1,5-7,9,14,17,18,20 page 73-101	1,11,34, 36
X	VON SEGGERN DAN ET AL., : "An adenoviral gene therapy vector deleted for E1, E3 and fiber: Structure and infectivity of fiberless particles" CANCER GENE THERAPY, vol. 5, no. 6, December 1998 (1998-12), page s14 XP000909076 abstract: P-39D	24-26, 31-33, 36, 40-45, 50-58, 60-63, 65-78

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/00265

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HARDY S ET AL: "CONSTRUCTION OF ADENOVIRUS VECTORS THROUGH CRE-LOX RECOMBINATION" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 71, no. 3, 1 March 1997 (1997-03-01), pages 1842-1849, XP000670223 ISSN: 0022-538X the whole document	76-85
X	FALGOUT B AND KETNER G: "Characterization of adenovirus particles made by deletion mutants lacking the fiber gene " JOURNAL OF VIROLOGY, vol. 62, XP000909209 the whole document	31,38, 42,44
A	STEVENSON S C ET AL: "HUMAN ADENOVIRUS SEROTYPES 3 AND 5 BIND TO TWO DIFFERENT CELULAR RECEPTORS VIA THE FIBER HEAD DOMAIN" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 69, no. 5, May 1995 (1995-05), pages 2850-2857, XP002911347 ISSN: 0022-538X the whole document	6-8,16, 17
A	PARKS R J ET AL: "A HELPER.DEPENDENT ADENOVIRUS VECTOR SYSTEM: REMOVAL OF HELPER VIRUS BY CRE-MEDIATED EXCISION OF THE VIRAL PACKAGING SIGNAL" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, no. 24, 26 November 1996 (1996-11-26), pages 13565-13570, XP000617948 ISSN: 0027-8424 the whole document	80-82
P,X	ZUFFEREY R ET AL.,: "Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors" JOURNAL OF VIROLOGY, vol. 73, no. 4, April 1999 (1999-04), pages 2886-2892, XP000906913 cited in the application abstract	9,10
P,X	EP 0 892 047 A (HOECHST MARION ROUSSEL DE GMBH) 20 January 1999 (1999-01-20) SEQ.ID.N.36	10

-/--

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INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/EP 00/00265

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	VON SEGGERN DAN J ET AL., : " A helper-independent adenovirus vector with E1, E3, and fiber deleted: Structure and infectivity of fiberless particles." JOURNAL OF VIROLOGY, vol. 73, no. 2, February 1999 (1999-02), pages 1601-1608, XP000906914 cited in the application the whole document	1-53, 59-75
T	VON SEGGERN DAN J ET AL., : "Adenovirus vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells." JOURNAL OF VIROLOGY, vol. 74, no. 1, January 2000 (2000-01), page 354-362 XP000906911 the whole document	1,9-13, 16-19, 25-29, 50-75,90

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INTERNATIONAL SEARCH REPORT

..ternational application No.
PCT/EP 00/ 00265

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 56-58 and 72 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir. ational Application No
PCT/EP 00/00265

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813499 A	02-04-1998	AU 4624197 A	17-04-1998
		EP 0937150 A	25-08-1999
WO 9737220 A	09-10-1997	US 5866341 A	02-02-1999
		AU 717289 B	23-03-2000
		AU 2661997 A	22-10-1997
		EP 0801307 A	15-10-1997
WO 9817783 A	30-04-1998	US 5994132 A	30-11-1999
		AU 5152998 A	15-05-1998
		EP 0935648 A	18-08-1999
WO 9500655 A	05-01-1995	AU 687829 B	05-03-1998
		AU 7118494 A	17-01-1995
		CA 2166118 A	05-01-1995
		EP 0705344 A	10-04-1996
		US 5919676 A	06-07-1999
WO 9639530 A	12-12-1996	US 5756283 A	26-05-1998
		AU 715533 B	03-02-2000
		AU 6277996 A	24-12-1996
		CA 2222880 A	12-12-1996
		EP 0835321 A	15-04-1998
		JP 11507240 T	29-06-1999
EP 0892047 A	20-01-1999	DE 19729211 A	14-01-1999
		DE 19805371 A	12-08-1999
		AU 7507698 A	21-01-1999
		BR 9802360 A	05-10-1999
		CA 2237158 A	09-01-1999
		CN 1209436 A	03-03-1999
		CZ 9802149 A	13-01-1999
		HU 9801511 A	28-05-1999
		JP 11235189 A	31-08-1999
		PL 327385 A	18-01-1999